JUNE 10, 1947

THE JOURNAL OF NUTRITION

VOLUME 33 NUMBER 6

GEORGE R. COWGILL, *Editor*
Yale Nutrition Laboratory, 333 Cedar Street,
New Haven 11, Conn.

EDITORIAL BOARD

ROLAND M. BETHKE — F. C. BING — WALTER C. RUSSELL
FRED A. HITCHCOCK — W. J. DANN — H. J. DEUEL, JR.
E. M. NELSON — S. LEPKOVSKY — HELEN T. PARSONS
O. A. BESSEY — E. W. MCHENRY — V. P. SYDENSTRICKER

Official Organ of the American Institute of Nutrition
PUBLISHED MONTHLY BY
THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY
PHILADELPHIA 4, PA.
Price, $5.00 per volume, Domestic; $5.50 per volume, Foreign

Entered as second-class matter January 20, 1934, at the post office of Philadelphia, Pa., under the Act of March 3, 1879. Copyright 1947. The Wistar Institute of Anatomy and Biology. All rights reserved.

Publications of The Wistar Institute

JOURNAL OF MORPHOLOGY

Devoted to the publication of original research on animal morphology and physiology, including cytology, protozoology, and the embryology of vertebrates and invertebrates. Articles do not usually exceed 50 pages in length.

Issued bimonthly, 2 vols. annually: $20.00 Domestic, $21.00 Foreign, per year.

THE JOURNAL OF COMPARATIVE NEUROLOGY

Publishes the result of original investigations on the comparative anatomy and physiology of the nervous system.

Issued bimonthly, 2 vols. annually: $15.00 Domestic, $16.00 Foreign, per year.

THE AMERICAN JOURNAL OF ANATOMY

Publishes the results of comprehensive investigations in vertebrate anatomy — descriptive, analytical, experimental.

Issued bimonthly, 2 vols. annually: $15.00 Domestic, $16.00 Foreign, per year.

THE ANATOMICAL RECORD

Organ of the American Association of Anatomists and the American Society of Zoologists

For the prompt publication of concise original articles on vertebrate anatomy, preliminary reports; technical notes; critical notes of interest to anatomists and short reviews of noteworthy publications.

Issued monthly, 3 vols. annually: $22.50 Domestic, $24.00 Foreign, per year.

THE JOURNAL OF EXPERIMENTAL ZOOLOGY

Publishes papers embodying the results of original researches of an experimental or analytical nature in the field of zoology.

Issued 9 times a year, 3 vols. annually: $22.50 Domestic, $24.00 Foreign, per year.

AMERICAN JOURNAL OF PHYSICAL ANTHROPOLOGY

Organ of the American Association of Physical Anthropologists

Publishes original articles on comparative human morphology and physiology as well as on the history of this branch of science and the techniques used therein. In addition it gives comprehensive reviews of books and papers, an annual bibliography, and informal communications.

Issued quarterly, 1 vol. annually: $6.00 Domestic, $6.50 Foreign, per year.

JOURNAL OF CELLULAR AND COMPARATIVE PHYSIOLOGY

Publishes papers which embody the results of original research of a quantitative or analytical nature in general and comparative physiology, including both their physical and chemical aspects.

Issued bimonthly, 2 vols. annually: $10.00 Domestic, $11.00 Foreign, per year.

THE JOURNAL OF NUTRITION

Organ of the American Institute of Nutrition

Publishes original researches in the field of nutrition and occasional reviews of literature on topics with which the journal is concerned.

Issued monthly, 2 vols. annually: $10.00 Domestic, $11.00 Foreign, per year.

THE AMERICAN ANATOMICAL MEMOIRS

Publishes original monographs based on experimental or descriptive investigations in the field of anatomy which are too extensive to appear in the current periodicals. Each number contains only one monograph. List of monographs already published, with prices, sent on application.

ADVANCE ABSTRACT CARD SERVICE

Every paper accepted for publication in one of the above periodicals is accompanied by the author's abstract. The abstract and the complete bibliography reference to the paper as it will eventually appear is printed on the face of a standard library catalogue card. This Advance Abstract Card Service is issued promptly, in advance of the journal containing the paper, and is offered in three styles.

Prices per year $2.00, $2.50 and $3.00, postpaid.

These publications enjoy the largest circulation of any similar journals published.

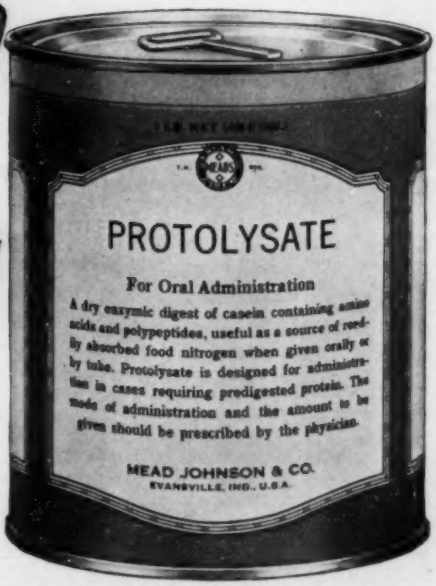
THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY

WOODLAND AVENUE AND THIRTY-SIXTH STREET, PHILADELPHIA 4, PA.

1000 cc. flasks
500 cc. flasks
125 cc. flasks
for hospitals

COUNCIL-ACCEPTED
AMIGEN
for parenteral administration of amino acids

The function of Amigen and Protolysate is to supply the amino acids essential for nutrition. Both can be given in place of protein when protein cannot be eaten or digested, or in addition to protein when the protein intake is insufficient. Administered in adequate amounts, they prevent wastage of protein, restore previous losses, or build up new body protein.


PROTOLYSATE
For Oral Administration
A dry enzymic digest of casein containing amino acids and polypeptides, useful as a source of readily absorbed food nitrogen when given orally or by tube. Protolysate is designed for administration in cases requiring predigested protein. The mode of administration and the amount to be given should be prescribed by the physician.
MEAD JOHNSON & CO.
EVANSVILLE, IND., U.S.A.

1 lb. cans at drug stores

COUNCIL-ACCEPTED
PROTOLYSATE
for oral and tube feeding of amino acids

Like Amigen, Protolysate is an enzymic digest of casein and consists of amino acids and polypeptides. Like Amigen, Protolysate supplies the nitrogen essential for maintenance, repair and growth.

Unlike Amigen, which may be employed both orally and parenterally, Protolysate is designed only for oral use.

MEAD JOHNSON & CO., EVANSVILLE 21, INDIANA

There is no shortage now of AMIGEN for parenteral use. There is no shortage now of PROTOLYSATE for oral use.

the unique essential amino acid

LYSINE ALONE—of the amino acids essential for man—cannot be synthesized from its immediate precursors within the body. Man's lysine requirement, therefore, must be met by his food intake.

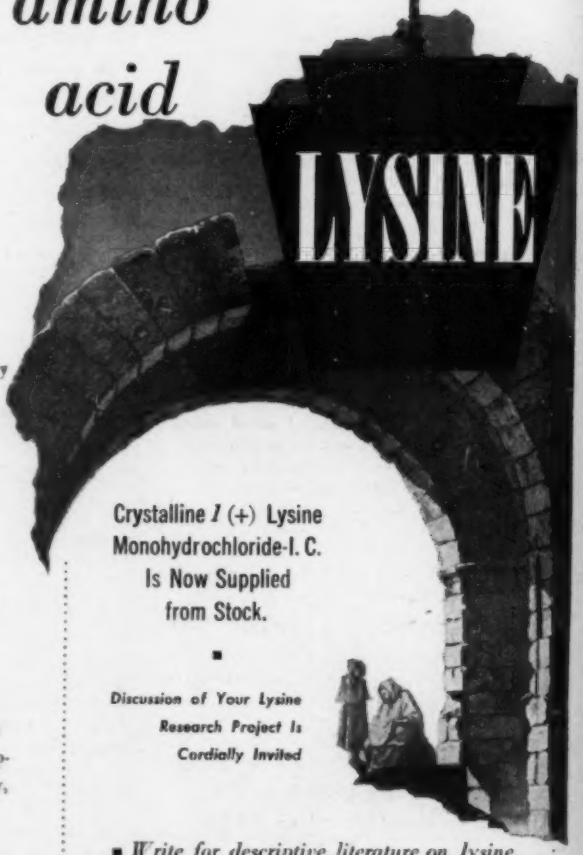
■ Lysine is seriously lacking in certain popular foods.

■ The *l* (+) isomer is the one form of lysine that is completely utilizable as a dietary supplement. It is as effective as lysine bound in the protein molecule.

■ The medical and nutritional aspects of these facts merit further biochemical and laboratory exploration.

■ Crystalline *l* (+) Lysine is now available for study of its clinical effects—as a dietary supplement—on growth, pregnancy, lactation, and convalescence.

■ *l* (+) Lysine merits investigation as a fortifier of processed foods and packaged cereals in which the bound-lysine may have been destroyed or inactivated by high temperatures.



Crystalline *l* (+) Lysine Monohydrochloride-I. C. Is Now Supplied from Stock.

■

Discussion of Your Lysine Research Project Is Cordially Invited

■ *Write for descriptive literature on lysine.*

Interchemical Corporation

Biochemical Division

UNION—NEW JERSEY

PROTEIN CHEMISTS—MANUFACTURERS OF AMINO ACIDS

Notes of interest from Borden's Quality Control Labs.

Ice Cream—the flexible food

Ice cream, long regarded as the great American dessert, is now clearly established as a versatile food *highly valuable to the normal diet.*

EASILY DIGESTIBLE

To begin with, ice cream is *highly digestible.* This is not only because its fat content is thoroughly homogenized—but also because 80% to 90% of its proteins are derived from milk—and milk proteins are the most easily and completely digested of all food proteins.

NOURISHMENT VALUE OF ⅓ PINT

From the standpoint of actual nourishment value, ice cream contributes much to the normal diet. For example, when a moderately active man eats an average portion of ice cream (⅓ pint) he satisfies 15.7% of his calcium needs, 10.3% of his vitamin A requirements, and 9.5% of his essential but often inadequately supplied riboflavin requirements.

LOW IN CALORIES

Yet, since it is relatively *low in calories,* containing about a fourth less

LOW CALORIC CONTENT

calories than apple pie and a third less than gingerbread, it is a good recommendation whenever a *low caloric diet* is indicated.

On the other hand, when a *high caloric diet* is in order, ice cream is useful served with cake, pie, rich sauces, and cookies, to increase the other essential nutrients along with the calories.

NEW BOOKLET AVAILABLE

To supply you with more detailed information about ice cream, The Borden Company has prepared an interesting and helpful new booklet, "In Sickness and in Health."

Written especially for doctors and dietitians, it gives a comprehensive analysis of ice cream as a food, and provides an extensive reference section on present-day practices on the use of ice cream in many types of illnesses and during convalescence. It is the first time that such a complete nutritional review has been compiled on the subject.

Write for your copy of "In Sickness and in Health" to Dept. NU, address below.

A WORD ABOUT BORDEN'S

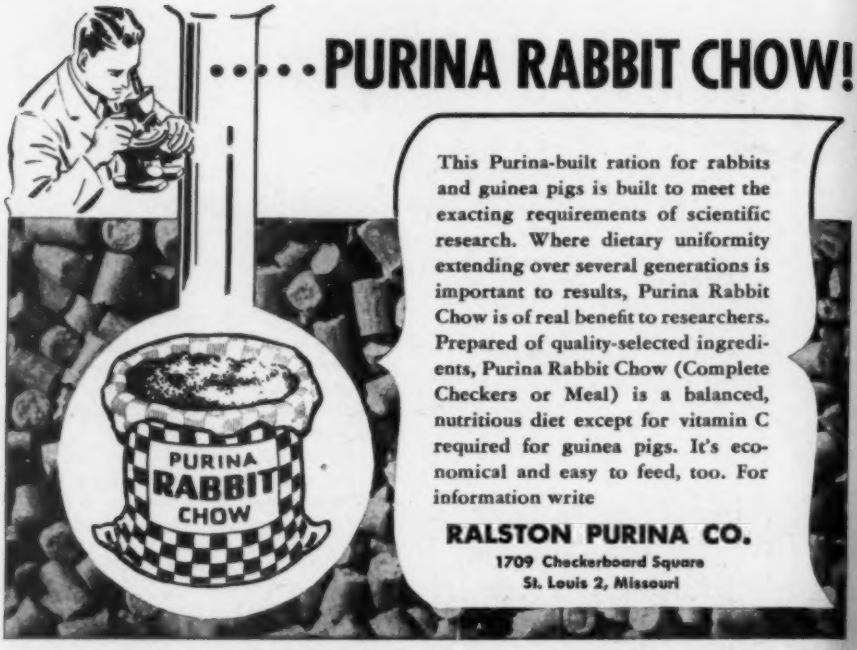
All ingredients used in Borden's Ice Cream are subject to rigid sanitary inspections, and every possible care is exercised to maintain the highest degree of quality control in every Borden Ice Cream plant. This is done so that Borden's Ice Cream will always be *a product you can always trust.*

BETTER NUTRITION THROUGH MILK RESEARCH AND QUALITY CONTROL

The *Borden* Company

350 Madison Ave., New York 17, N.Y.

MANUFACTURERS AND DISTRIBUTORS OF BIOLAC, BETA LACTOSE, KLIM & DRYCO, BORDEN'S ICE CREAM, HEMO, EVAPORATED MILK, INSTANT COFFEE, FRESH MILK & CHEESE



....PURINA RABBIT CHOW!

This Purina-built ration for rabbits and guinea pigs is built to meet the exacting requirements of scientific research. Where dietary uniformity extending over several generations is important to results, Purina Rabbit Chow is of real benefit to researchers. Prepared of quality-selected ingredients, Purina Rabbit Chow (Complete Checkers or Meal) is a balanced, nutritious diet except for vitamin C required for guinea pigs. It's economical and easy to feed, too. For information write

RALSTON PURINA CO.
1709 Checkerboard Square
St. Louis 2, Missouri

ROCKLAND

SMALL ANIMAL

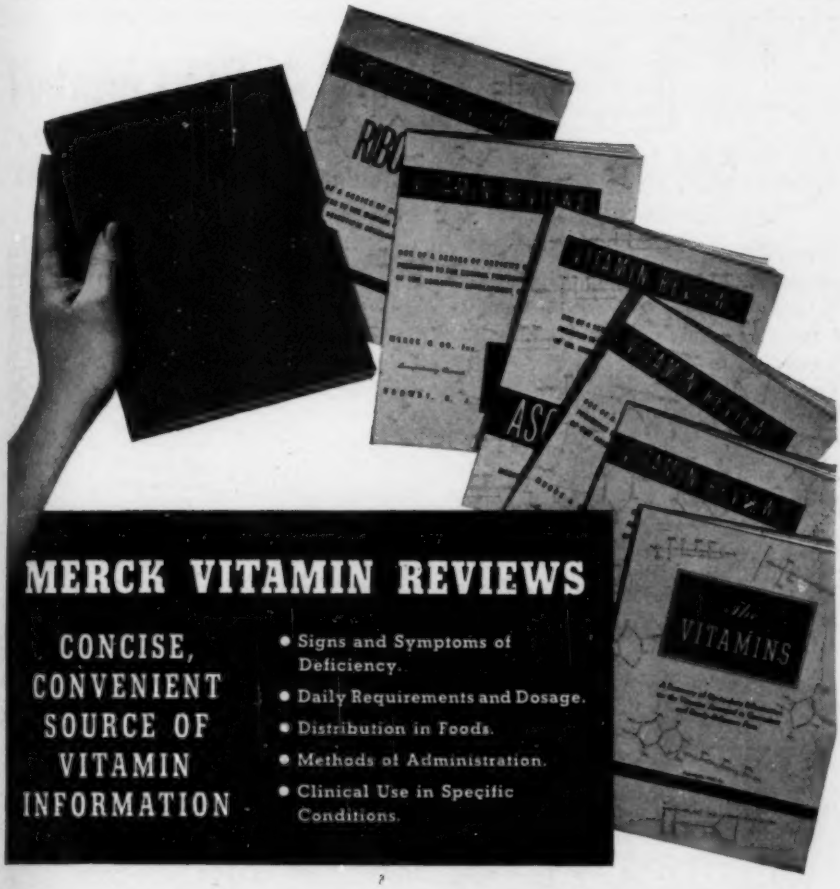
STOCK DIETS

are specie specific, complete and in pellet form.

ROCKLAND MOUSE DIET
ROCKLAND RAT DIET (complete and 'D' free)
ROCKLAND GUINEA PIG DIET
ROCKLAND RABBIT RATION

Milled and distributed solely by

ARCADY FARMS MILLING CO.
223 WEST JACKSON BLVD. CHICAGO, ILL.
Authorized Dealers in All Principal Cities


MERCK VITAMIN REVIEWS
CONCISE, CONVENIENT SOURCE OF VITAMIN INFORMATION
• Signs and Symptoms of Deficiency.
• Daily Requirements and Dosage.
• Distribution in Foods.
• Methods of Administration.
• Clinical Use in Specific Conditions.
the VITAMINS

A limited number of complete sets of these informative booklets has been gathered in a convenient slip-cover container, designed for ready reference. These are available as long as the supply lasts. The coupon is for your convenience.

MERCK & CO., Inc., RAHWAY, N. J.
Manufacturing Chemists

Please send me a complete set of *Merck Vitamin Reviews* in convenient slip-cover container.

Name.....
Street.....
City.....State.....

Reg. U.S. GBI Pat. Off.

CRYSTALLINE VITAMINS

Essential as Standards and Supplements for Biological Research

Alpha Carotene	Ca Pantothenate
Beta Carotene	Inositol
Carotene (90b—10a)	2-Methyl Naphthoquinone
Vitamin A	Niacin
Ascorbic Acid	Niacin Amide
Alpha Tocopherol	p-Aminobenzoic
Biotin	Pyridoxine HCl
Choline Chloride	Riboflavin

Thiamin Chloride

Conveniently Packed—Economically Priced
Write for Price List No. 677

GENERAL BIOCHEMICALS, INC.

61 LABORATORY PARK CHAGRIN FALLS, OHIO

Laboratory Pre-Tested for Microbiological Uses

CASEIN HYDROLYSATES

"Vitamin Free" Casein Hydrolysate GBI—(acid hydrolyzed) for U.S.P. and other standard assays for B-complex vitamins, amino acids and bacterial nutrition studies. 100 cc. serum bottles—$18.00 per dozen.

"Special" Casein Hydrolysate GBI—same as above product but more economical to use for all those procedures where its content of trace quantities of niacin will not interfere. 100 cc. serum bottles—$15.00 per dozen.

Related GBI Products

Special Media, Crystalline Vitamins and Amino Acids. (Enzymatic) Hydrolysates of Lactalbumin and Casein—powder form.

Write for prices and information

GENERAL BIOCHEMICALS, INC.

61 LABORATORY PARK, Reg. U.S. GBI Pat. Off. CHAGRIN FALLS, OHIO

INTERNATIONAL EXHIBITION OF BIOLOGICAL PHOTOGRAPHY

Auspices of
Biological Photographic Association

Exhibit Date:
September 10 – 27, 1947

Place:
Rundel Building,
Rochester Public Library,
Rochester, New York

Closing Date for Entries:
August 1, 1947

Membership in the Biological Photographic Association is not a prerequisite for participation in the Exhibition

Annual Meeting
of the
Biological Photographic Association

Rochester, New York

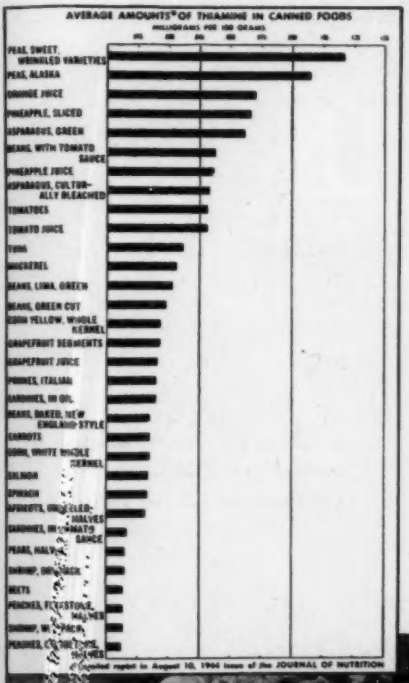
September 10 – 13, 1947

For further information communicate with

MISS ANNE SHIRAS
Secretary,
Biological Photographic Association
SCHOOL OF MEDICINE,
UNIVERSITY OF PITTSBURGH
PITTSBURGH, PA.

IT'S NET FIGURES THAT COUNT!

FACTS now available! Average net amounts of 12 nutrients in the most frequently consumed canned foods



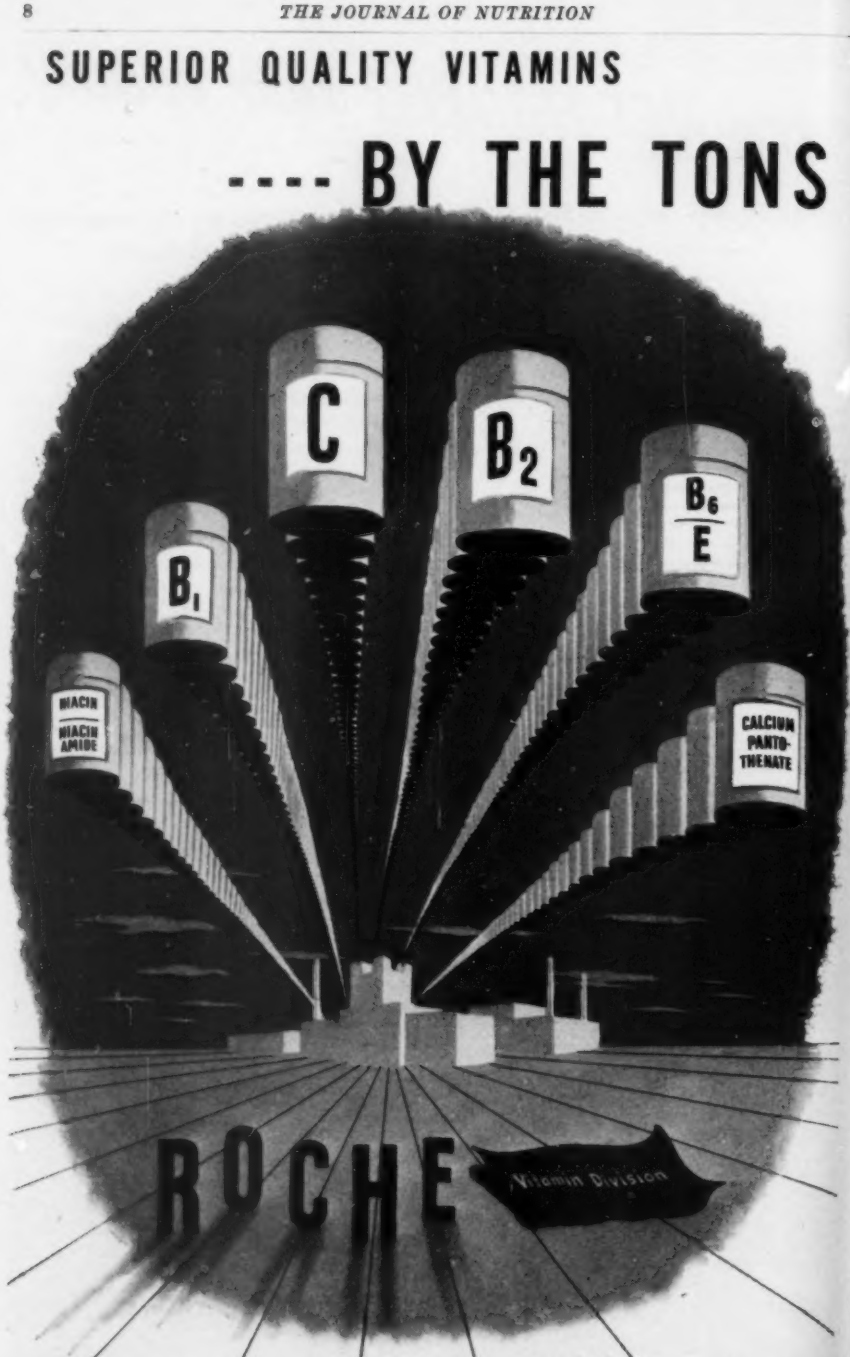
Nutrition tables have long been available for all of the foods covered in this series of advertisements. However, as you know, the figures usually quoted in such tables are *gross* figures for raw, uncooked foods. Such figures do not take into consideration widely varying deductions for losses occurring in transit from field to market, to kitchen

Not so in the case of the typical figures shown for thiamine, in the chart on the left. These figures, based on research at 5 universities, are actual, *net* values in cooked, ready-to-eat-or-heat food packed in cans . . . real proof of high vitamin and mineral retention throughout the canning process.

We think the facts justify your sincere recommendation of canned foods as a source of low-cost nutrition. A series of 12 charts on the actual nutritional values of popular commercially canned foods is available in booklet form. For your copy, please address: Can Manufacturers Institute, Inc., 60 E. 42nd St., New York 17, N. Y.

SUPERIOR QUALITY VITAMINS

---- BY THE TONS



HOFFMANN-LA ROCHE, INC., NUTLEY 10, NEW JERSEY

METABOLISM OF WOMEN DURING THE REPRODUCTIVE CYCLE

XI. VITAMIN C IN DIETS, BREAST MILK, BLOOD AND URINE OF NURSING MOTHERS [1]

BERTHA MUNKS, MILDRED KAUCHER, ELSIE Z. MOYER, MARY ELLEN HARRIS AND ICIE G. MACY

Research Laboratory, Children's Fund of Michigan, Detroit

(Received for publication January 30, 1947)

As part of a comprehensive investigation of the vitamin and mineral composition of human milk and the metabolism of women during the reproductive cycle we have determined nursing mothers' intakes of vitamin C, their secretion of the vitamin in milk, excretion in the urine, and the amounts in the cord blood as well as the levels in the blood of both mother and child. Other investigations have not included analyses of 24-hour collections of food, milk, and urine during the 10 days of the puerperium and at intervals during mature milk production, accompanied by determinations of vitamin C in the blood of the same subjects and their infants. The intake of vitamin C provided for the infant by his mother's milk will be discussed in another paper.

EXPERIMENTAL

The subjects of the investigation were multiparas with medical records of good or excellent health and of having successfully nursed their other children. Every effort was

[1] The investigation represented in part by this paper was partially supported by a grant from The Nutrition Foundation, Inc., and was made possible by the cooperation of Dr. J. P. Pratt, Chief of the Department of Obstetrics, Dr. B. M. Hamil, Department of Pediatrics, Elizabeth Moran, Director of Nurses, and Annie Lou Wertz, dietitian, all of the Henry Ford Hospital, Detroit.

Copyright, 1947
The Wistar Institute of Anatomy and Biology.
All rights reserved.

made to standardize any procedure involving controllable factors. Vitamin C was determined by analysis of samples representing the total intake of vitamin-C-containing foods for 24-hour periods, thus obtaining quantitative estimations of the amounts ingested. Qualitatively the diets were similar for all women during all 5-day periods (Kaucher et al., '45, '46); however there were differences in consumption owing to variations in appetites and food composition. Analyses of complete 42-hour collections of milk and urine obtained during the first 10 days postpartum and during each day of 5-day periods at intervals during lactation avoided portrayal of diurnal changes in concentration in the results. In addition, vitamin C was determined in placental tissue (Pratt et al., '46), in blood from the umbilical cord and in capillary blood obtained from both mother and infant on the first and tenth days after delivery, and the first and last days of 5-day periods of study. To obtain comparable data, the blood samples were taken from mother and child in the morning after expression of the milk but before breakfast.

Analyses of food, milk and urine were made by the method of Roe and Kuether ('43); of blood, by the method of Farmer and Abt ('36). Preceding publications have given details of selection of subjects (Macy, Williams, Pratt and Hamil, '45), diet and procedures of sampling and preparation of food composites and milk (Kaucher et al., '45), the method of manually expressing milk (Davies, '45) and collection and analysis of urine (Roderuck, Williams and Macy, '46). The concentration of vitamin C in immature and mature human milk has been presented (Munks et al., '45).

RESULTS

The volumes of milk secreted and urine excreted by 4 women during each of the first 10 days postpartum, the intakes of vitamin C per day and the amounts of vitamin C in the immature milk and in the urine for each day are given in table 1.

Within each 5-day period there were wide variations from day to day in vitamin C intake, depending upon the kinds of

TABLE 1

Vitamin C intake, secretion and excretion during first 10 days postpartum.

SUBJECT	INTERVAL POSTPARTUM	VOLUME		VITAMIN C		
		Milk	Urine	Intake	Milk	Urine
	days	*ml*	*ml*	*mg*	*mg*	*mg*
V.K.	1	9	3086	148	..	24.9
	2	90	3651	112	..	15.7
	3	484	2596	77	50	13.4
	4	547	2427	128	54	11.6
	5	560	2331	83	50	12.4
	6	663	2249	126	60	10.3
	7	781	3380	115	61	8.3
	8	775	3840	63	70	9.6
	9	794	3115	176	70	9.8
	10	797	3328	51	61	3.5
V.L.	1	30	1757	138	1	23.9
	2	56	2686	98	4	14.9
	3	353	2759	86	25	11.4
	4	794	1823	204	50	13.4
	5	844	1847	98	55	14.1
	6	955	2710	200	63	18.2
	7	1047	2841	161	69	14.2
	8	1098	2066	87	76	14.2
	9	1118	2151	183	73	11.8
	10	1200	1669	99	83	9.2
M.	1	35	2182	216	2	8.9
	2	385	1386	147	23	6.4
	3	870	1519	96	44	8.2
	4	1011	1201	201	56	6.5
	5	1121	1283	114	62	8.5
	6	1125	1172	169	51	4.7
	7	1287	1161	151	75	6.4
	8	1136	1520	92	55	6.6
	9	1258	....	254	83	..
	10	1336	1553	59	87	7.5
V.S.	1	6	1869	204		34.7
	2	92	1874	98	8	24.9
	3	420	2887	98	37	28.2
	4	600	2063	207	49	35.4
	5	697	2065	112	54	26.4
	6	736	1502	156	54	22.8
	7	818	2524	154	65	22.0
	8	837	2237	98	67	20.9
	9	932	1984	196	76	19.2
	10	924	2287	89	65	15.0

foods included in the diet for the different days. The lowest vitamin C intake for any day was 51 mg, the highest was 254 mg. The average daily consumption for the 10 days was 134 mg, an amount lower than the 150 mg recommended by the Food and Nutrition Board of the National Research Council ('45). However, it is important in evaluating the data on vitamin C to remember that the values represent intakes determined by analysis of food *as eaten*, by 1 method of determination, whereas the Recommended Allowances are based upon data obtained by various analytical methods and were planned to include ample amounts as "safety factors."

It is evident that the amounts of vitamin C secreted in the milk each day were not related to the daily fluctuations in intake, but paralleled the increased production of milk, secondarily influenced by the slightly augmented ascorbic acid concentration (Munks et al., '45). The high excretions of vitamin C in the urine by 3 of the mothers after delivery, followed by decreases of 50% or more within the first 10 days postpartum, parallel the decreased urinary excretions of vitamin C reported for newborn infants (Hamil et al., '47) and support the belief that the tissues of both mother and fetus require greater concentrations of vitamin C for saturation during pregnancy than during lactation.

Subject V.S., who had been taking 100 mg of ascorbic acid per day throughout pregnancy, in addition to an excellent diet and supplements of other vitamins (Roderuck, Williams and Macy, '46; Roderuck, Coryell, Williams and Macy, '46) secreted milk with the highest concentration of vitamin C each day of the puerperium and also excreted greater amounts in urine (15.0 to 35.4 mg) than did any of the other women. Subject J.M., whose milk flow was established more rapidly than that of the other 3 women and reached the highest level during the first 10 days, excreted much less vitamin C in urine during the first few days after delivery, but continued to excrete at approximately the same level regardless of the increased secretion in milk.

Despite the similarity of the women's average daily intakes during each 5-day period and the relationship between volume of milk produced and its vitamin C content, the data in table 1 do not indicate a relationship between volume of milk or its vitamin C content and excretion of the vitamin in urine. Nor was urine volume a factor in excretion of vitamin C, which ranged from 3.5 mg in 3328 ml to 35 mg in 1869 ml of urine. Several possibilities are suggested by the urine values. If the level of tissue saturation required during lactation is lower than that demanded by pregnancy, the decline of over 50% in the daily excretion of vitamin C by 3 women during the first 10 days postpartum may represent an "unloading" from the maternal tissues. If this is true it would indicate that the tissues of subject J.M. had not been saturated during the prepartum period or that the amount which she might have "unloaded" after delivery was expended during labor and childbirth. That the woman's prepartum nutritional status was not poor is evidenced by the medical records, by her quick establishment of lactation, and the secretion in milk of greater amounts of vitamin C per day than any other woman. The fact that J.M. maintained a level of excretion in urine ranging from 5 to 9 mg of vitamin C per day also indicates that the decreased excretions by the other women are not attributable to the demands of increased milk production. The possibility that J.M. was excreting required minimal amounts in urine is doubtful because the urinary vitamin C of V.K. and V.L. declined to comparable levels by the tenth day postpartum.

The vitamin C contents of the serum of the cord blood and the blood of mother and infant, shown in table 2, contribute additional information on the utilization of the vitamin. Except for subject L.F., the cord blood of all women had a greater concentration of vitamin C than did blood taken from mothers and infants on the first day postpartum. In all but one instance (J.M.) blood vitamin C levels for both mother and child were lower on the tenth than on the first day postpartum, and the levels in the infant's blood were as high or

higher than those in their mothers' blood. The cord blood values ranged from 0.4 to 2.2 mg vitamin C per 100 ml. The infant blood contained 0.2 to 2.2 mg and the maternal blood from 0.2 to 1.4 mg per 100 ml during the first 24 hours postpartum. These values are within the range given by other workers. On the tenth day postpartum the infant and maternal vitamin C blood serum concentration ranged from 0.4

TABLE 2

Blood serum vitamin C during the first 10 days postpartum. (mg/100 ml.)

SUBJECT	CORD	INTERVAL POSTPARTUM	MOTHER	CHILD
		days		
V.K.	1.3	1	0.6	1.0
		10	0.4	0.8
V.L.	2.2	1	0.6	0.7
		10	0.4	0.4
J.M.	0.4 [1]	1	0.2	0.2
		10	0.1	0.5
V.S.	1.5	1	0.3	1.0
		10	0.3	0.6
L.F.	1.6	1	1.4	2.2
		10	0.8	1.9
V.G.	..	1	1.3	2.2
		10	0.6	1.2
C.O.	1.5	1	0.7	..
		10	0.1	0.4

[1] Placental.

to 1.9 mg and from 0.1 to 0.8 mg, respectively. The values for cord, mothers' and infants' blood were within the ranges, respectively, found for 24 other healthy mothers and their infants during the first week of life (Hamil et al., '47).

The values in tables 1 and 2, supported by the values presented earlier (Hamil et al., '47) for vitamin C in cord and maternal blood during the first week postpartum, emphasize the inconclusiveness of determinations of vitamin C levels in either blood or urine, or both, as an index of nutritional status with respect to vitamin C during lactation. Among women receiving diets undoubtedly superior to those of most mothers

during the puerperium, with appetite-inhibiting factors eliminated (coffee, tea, candy, etc.), vitamin C in the maternal blood and urine may be at levels generally considered to indicate scurvy (J.M.), yet not interfere with secretion of large volumes of milk containing ample amounts of the vitamin. Levels of vitamin C in the blood may also be low in subjects known to have been "saturated" before delivery (V.S.) although large amounts are being excreted in the urine. Recent work indicates that the ascorbic acid content of the white blood cells parallels the retention and may be a reliable index of total body concentration of ascorbic acid (Lowry et al., '47) in normal adults. Whether similar results would be obtained with nursing mothers is not known.

The variations in the vitamin C contents of milk, blood, and urine from healthy mothers receiving excellent diets containing 51 to 254 mg of vitamin C per day, determined under conditions as closely controlled as possible, indicate a wide range of normal, individual physiologic differences. Data obtained under the conditions of the study are not comparable to those procured with subjects receiving low intakes of vitamin C from inferior diets or high intakes from supplemental sources; nor can they be compared closely with results obtained with non-lactating women or those obtained with dietary intakes calculated from tables of food values. Further research is needed to clarify the influences exerted by the many factors involved in the vitamin C metabolism of pregnant and nursing women. Among these are the possibility of fetal synthesis of the vitamin, physiologic adjustment of the maternal body from conditions of pregnancy to those of lactation, including possible reorganization of the glands of internal secretion, the level of requirement for the vitamins as opposed to maximum level of storage (saturation), and differences in the requirements before pregnancy, during gestation, and the lactation period.

The intakes of vitamin C by 6 women during 13 5-day periods in which they were secreting mature milk, are given in table 3, with the volumes of milk secreted and urine excreted

TABLE 3

Daily vitamin C intakes, secretion in mature milk and excretion in urine.

SUBJECT	INTERVAL POSTPARTUM	VOLUME		VITAMIN C		
				Daily total		
		Milk	Urine	Intake	Milk	Urine
	days	*ml*	*ml*	*mg*	*mg*	*mg*
V.G.	78	683	1663	178	36	4.8
	79	846	1359	112	44	4.3
	80	923	1419	70	51	5.4
	81	874	844	194	52	6.5
	82	913	1120	86	49	6.2
	161	759	989	94	39	5.4
	162	848	801	118	43	7.3
	163	912	813	75	46	5.8
	164	985	1005	130	46	8.2
	165	1000	1359	108	51	2.0
	239	610	853	136	48	61.2
	240	624	731	112	48	34.5
	241	661	736	84	50	43.2
	242	738	958	104	41	47.5
	243	772	770	72	54	33.4
	302	414	1350	189		39.2
	303	389	1068	140	30	33.4
	304	383	949	120	30	30.3
	305	389	1099	171	30	42.0
	306	394	933	96	27	23.0
V.K.	95	544	3072	115	36	10.6
	96	615	3443	106	39	11.2
	97	701	2587	91	45	6.9
	98	691	3206	139	44	10.4
	99	684	1710	61	44	8.3
	144	272	2739	132	22	12.8
	145	315	1286	136	26	11.5
	146	343	1476	180	29	13.3
	147	323	827	155	25	13.5
	148	372	907	121	28	10.6
V.L.	68	736	3408	180	49	10.4
	69	743	4154	140	46	11.8
	70	802	2838	195	53	12.8
	71	833	1903	175	49	6.8
	72	829	1940	120	51	10.5

TABLE 3 (*continued*)

SUBJECT	INTERVAL POSTPARTUM	VOLUME		VITAMIN C		
				Daily total		
		Milk	Urine	Intake	Milk	Urine
	days	*ml*	*ml*	*mg*	*mg*	*mg*
V.L.	152	684	2457	227	47	68.9
	153	671	1970	145	43	26.9
	154	673	1637	105	43	23.9
	155	686	1484	218	44	46.8
	156	686	1232	85	39	17.2
J.M.	75	598	2517	153	27	6.3
	76	677	2081	110	29	6.4
	77	740	2155	171	29	0.0
	78	742	2646	157	34	7.6
	79	783	1925	100	38	4.0
	173	205	2993	183	12	5.0
	174	231	1922	156	13	3.6
	175	296	2089	79	16	4.0
	176	301	1169	129	16	4.2
	177	309	1841	108	15	5.1
B.S.	204	864	1630	158	32	6.0
	205	927	1350	133	35	7.2
	206	918	1231	79	37	6.3
	207	938	1219	200	41	6.2
	208	918	1352	78	40	5.9
	259	734	1665	146	37	7 6
	260	612	1299	95	29	7.0
	261	631	1037	76	30	7.7
	262	725	979	166	34	4.8
	263	678	1113	89	36	7.8
V.S.	70	234	1897	315	23	141.3
	71	276	1846	202	29	78.4
	72	334	2308	200	31	75.1
	73	315	1938	275	30	118.3
	74	361	1269	148	32	48.7

per 24 hours and their contents of the vitamin. During 10 of the periods the mothers' volumes of milk were increased 54 to 241 ml per day as the result of regular and complete emptying of the breasts by manual expression (Macy, Hunscher, Donelson and Nims, '30). Also for some of the women there

was an improvement of diet during the experimental periods. Although the diets for all 5-day periods were comparable, qualitatively, and appetites were satisfied by proportionate adjustment of all foods except milk, the vitamin C intakes as determined by analysis varied greatly owing to differences in concentration of the vitamin in the same foods. For example, V.L., J.M., and V.S. received diets of equal amounts, respectively, of the same foods (table 3). Calculated from the literature their average daily intakes of vitamin C during the 5-day periods ranged from 166 to 169 mg. The analyses for the different intervals of study averaged from 131 to 228 mg per day.

There were large differences in the vitamin C intakes for the 5 days of each study period owing to inclusion of foods with high contents of the vitamin in some of the menus. However, the concentration of vitamin C in mature milk was quite constant, averaging 5 mg per 100 ml (Munks et al., '45), and in general the daily secretion of the vitamin paralleled changes in milk volume. Although for the well-nourished mothers no direct relationship was found between the variable vitamin C intakes and the amount secreted in the 24-hour collections of milk, other work has shown vitamin C secretion to be generally dependent on diet (King, '38; Smith, '38; Escudero and Pierangeli, '43). Escurdero and Pierangeli have reported that with an intake of approximately 70 mg vitamin C per day from food during the last month of pregnancy and the colostral period, the colostrum averaged 2.2 mg % vitamin C. Women receiving 240 mg produced colostrum containing 5.5 mg per 100 ml. Selleg and King ('36) found that the relatively slow and limited rise in C value when patients received a special orange juice supplement provides an indication of a maximum and approximately optimum level of secretion, above which an excessive dietary intake results chiefly in a rapid urinary excretion without disturbing the lactation level. Such results emphasize the ample provision of vitamin C by the diets eaten by the nursing mothers in this study.

Levels of excretion in the 24-hour collection of urine were not related to differences in level of intake among the women of this study, nor to the vitamin C secretion in milk, again indicating that individual characteristics conditioned by prior nutritional status with respect to the vitamin were the major controlling factors. It has been shown that in addition to tissue saturation excretion of vitamin C in urine may be influenced by the individual's kidney threshold for the vitamin (Faulkner and Taylor, '38), by the fluid intake, and by environmental temperature and humidity (Shields and coworkers, '46) all of which were beyond the control of the present study. It is likely that other individual characteristics are involved, so it is not surprising to find wide variations in the urinary vitamin C among individuals and for the same individual at different times during lactation.

The concentrations of vitamin C in the blood serum of both the mothers and infants during the 5-day periods of mature milk production are given in table 4. The average values correspond for all determinations on mothers' blood during mature milk secretion (0.45 mg) and during the first 10 days postpartum (0.56), and the distributions are similar. The average of the values for the breast-fed infants when 2 to 10 months old (1.1 mg) approximates that for the first 10 days postpartum (1.0 mg). Fourteen of the samples from the mothers were obtained on the first days of study periods; 5 were obtained after the women had received the study diet for 5 days. The values for all 5 were the same or higher than those of the first days of study, respectively, attesting the adequacy of the study diet and perhaps indicating its superiority over their usual food intakes. However, this conclusion is precluded by the secretions in milk, the levels in the infants' blood, and by the excretions in the urine, especially those of V.S. who had been ingesting 100 mg of ascorbic acid per day with a good diet, prior to the study, who secreted milk with the highest concentration of the vitamin (10 mg per 100 ml), yet whose blood level on the first day (0.1 mg) was lower than any other value obtained and increased during the 5 days with

an intake undoubtedly lower than that of her previous supplemented diet.

It is evident that milk secretion requires that the requisite substances be supplied through the blood, either from the products of digestion or from the tissues. The best estimates

TABLE 4

Blood serum vitamin C during mature milk secretion. (mg/100 ml.)

SUBJECT	INTERVAL POSTPARTUM	MOTHER	CHILD
	days		
V.G.	78	0.3	0.5
	161	0.2	1.7
	239	0.6	0.8
	244 [1]	1.0	
	302	0.2	1.1
	307 [1]	0.6	..
V.K.	95	0.8	3.0
	144	0.7	0.9
V.L.	68	0.3	1.5
	152	0.8	1.4
	157 [1]	0.8	..
J.M.	75	0.2	0.7
	172	0.2	0.6
	177 [1]	0.6	..
B.S.	85	0.2	1.0
	204	0.2	0.8
	259	0.4	0.6
V.S.	70	0.1	0.8
	75 [1]	0.4	..

[1] After receiving study diet for 5 days.

are that 400 to 500 volumes of blood are required to produce 1 volume of milk (Kay, '45). Obviously, withdrawal from the blood parallels the rate of secretion, which is determined by the fullness of the gland. It is likely that, for vitamin C at least, the blood level is more easily replenished from the supply provided by food than by mobilization from body stores.

Whether the level of the vitamin in the blood of nursing mothers changes rapidly in response to emptying of the breasts and also varies in relation to the intervals between meals is being investigated.

The net gain or loss of vitamin C to the body, estimated by subtraction of the values for urine and milk from the intakes, indicates the amount of the vitamin used in metabolism and stored in the tissues, or the deficit which has been withdrawn from the tissues. The quantity of vitamin C excreted through the skin would seem inappreciable, since recent work has shown that at comfortable temperatures the loss may amount to 0.8 mg daily and even under conditions of induced profuse sweating the quantity is only 2.7 mg per day (Shields, et al., '46). In the 10-day puerperium the total quantities of vitamin C consumed by V.K., V.L., J.M., and V.S. were 1079, 1354, 1499 and 1412 mg, respectively (table 1). During the same period the women secreted into their milk, totals of 476, 499, 538 and 475 mg of vitamin C. Their urine contained 120, 145, 64 and 250 mg, leaving for metabolic function and tissue storage 483, 710, 897 and 687 mg, respectively. There were wide individual variations among these 4 women as shown by the range of 113 to 205 mg per day which was unaccounted for immediately following recovery from the trauma of labor and delivery, about twice the 25 to 50 mg daily observed in persons in a state of saturation (King, '38). It is conceivable that during this recuperation period there are appreciable changes in the acid-base equilibrium of the body. This is supported by evidence that the quantity of vitamin excreted in the urine may be varied merely by changing the acid-base balance of the food intakes (Hawley and co-workers, '36).

Twenty-seven of 64 daily determinations with women secreting mature milk showed more than 90 mg per day unaccounted for on days when the intakes were high (table 3), which indicates, perhaps, that the mothers were not saturated and the amount was dependent on the intake. The greatest amount unaccounted for was 166 mg per day, opposed to a loss of 15.4 mg per day from the mother's tissue stores. During

a 5-day study in the eighth month postpartum, V.G. had an average intake of 101.6 mg vitamin C and a secretion of 48.2 mg in the milk. These values are similar to those of the same woman when studied at 3 and 6 months, yet the excretion of vitamin C in the urine was 44 mg per day, over 8 times the average excretion during the earlier studies and leaving only 9.4 mg vitamin C per day unaccounted for. The blood serum vitamin C concentration was 0.6% on the first day of the study and increased to 1.0 mg % on the fifth day of the study (table 4). If the daily body requirement for vitamin C is 50 to 100 mg per day or if the mother's indispensable minimum requirement was 25 mg per day (King, '38) it is difficult to understand why she excreted an average of 44 mg vitamin C per day through the kidneys, unless she previously had a high vitamin C intake which continued to be excreted even after the intake was lowered. This phenomenon has been demonstrated by Harris, Ray and Ward ('33), Harris and Ray ('35) and Hess and Benjamin ('34), who found the urinary output to depend on both the immediate vitamin C intake and also on the post-nutritional history or state of "saturation."

When studied during the tenth month of lactation the same woman's average intake was 143.2 mg per day. The vitamin C excreted in the milk had dropped almost one-half, 29.2 mg per day, owing to decreased volume. Her urine excretion, 33.6 mg per day, still was higher than during the third and sixth months. The vitamin C unaccounted for had returned to the former range of 80 mg average per day. The need for this increased retention was shown by the blood serum concentration which was only 0.2 mg per 100 ml at the beginning of the study but increased to 0.6 by the fifth day (table 4).

A similar saturation was demonstrated by V.L. during her third and sixth months postpartum. The average intakes of vitamin C were 162 and 156 mg, secretion in the milk amounted to 49.6 and 43.2 mg, and the urine excretion averaged 10.5 and 36.7 mg of vitamin C per day, respectively. At the beginning of the study at 3 months the blood serum level was 0.3 mg

per 100 ml but at the beginning of the 5-day study at 6 months, when she was throwing off greater amounts of vitamin C in urine, her blood level was 0.8 mg of vitamin C per 100 ml. Large vitamin C excretions may occur even when the blood serum vitamin C is low, if the dietary intake is high. For example, V.S. in the third month of lactation excreted 92.4 mg of vitamin C per day, yet she had a blood serum level of only 0.1 mg % vitamin C on the first day of the study. Her average dietary intake was 228.0 mg vitamin C over the 5-day period and was the highest average intake during any 5-day study.

The amount of vitamin C which appears in the urine is also affected by the way in which the daily intake is distributed within the 24 hours. Widenbauer and Kühner ('37) and Ralli, Friedman and Sherry ('39) have emphasized the importance of dividing the intake into small doses throughout the day. Todhunter and Robbins ('40) have estimated the minimum daily intake of vitamin C required to maintain the tissues in a state of complete saturation to be 1.6 to 1.7 mg per kg of body weight per day for college women. Ralli, Friedman and Sherry ('39) concluded from their experiments that 100 mg per day be suggested as the requirement of women for saturation. With the exception of a woman who weighed 73.5 kg, the daily requirement of the lactating women in this study would have been 100 mg per day or less by this criterion. If one adds the amounts of vitamin C secreted in milk to the amounts calculated from their weights to be the mother's requirements, one obtains an estimated daily requirement for lactating women ranging from 106.8 to 174.1 mg of vitamin C per day. During the first 10 days postpartum the vitamin C intakes of 2 women met the estimated dietary requirement. For the woman who weighed 73.5 kg the estimated requirement was greater than the average daily intake during the first 10 days postpartum. During the third month of lactation the diet supplied this theoretical requirement for saturation in 3 of 5 women.

One of the lowest blood values, 0.1 mg vitamin C per 100 ml, was recorded for a woman who secreted the highest concentra-

tion of vitamin C in milk, 10 mg per 100 ml. Other women having blood serum values up to 0.8 mg per 100 ml did not secrete as great concentrations of vitamin C in the milk. One, V.G., was known to secrete over 7 mg per 100 ml in her milk when her blood serum vitamin C ranged anywhere from 0.2 to 1.3 mg per 100 ml. These data confirm the observation of Ingalls, Draper and Teel ('38) that there is no direct correlation between the blood serum vitamin C and the concentration in the milk. However, they state that "the fact that mothers on a diet relatively low in vitamin C will secrete in the milk amounts of ascorbic acid so large that the ascorbic acid content of the infant's plasma is higher than that of the mother's is indicative of an interesting biologic mechanism whereby the child receives the vitamin at the expense of its mother." For the lactating mother and the breast-fed infant vitamin C levels in the blood, as well as in urine, may be closely correlated with eating time. In this study blood samples were taken following collection of milk and urine at 6:00 A. M. It is possible that the low values for the mother's blood were the result of the demands for milk production during the preceding 12 hours of fasting. If this is correct then the supply in the blood would be replenished immediately after eating breakfast and the low fasting values would not indicate that the child receives vitamin C "at the expense of the mother."

Until requirements can be more clearly distinguished from "storage" or "saturation," it is obvious that for the physician nutrition during the nursing period is a problem similar to that stated for pregnancy by Lund ('45): ". . . the problem is the dietary needs of each patient. She is not to be treated according to the nutritional standards of any other person or groups of persons. She is an individual problem and it is the physician's duty to take a short, accurate dietary history and to see that he or some other qualified person explains to her the dietary problems of pregnancy." From the present study it seems that the Recommended Dietary Allowances ('45) of the Food and Nutrition Board of the National Research Council provide for adequate amounts of

vitamin C to meet normal requirements during lactation and prevent depletion of body stores, provided during pregnancy the nutritional status of the mother has been adequate with respect to the vitamin.

SUMMARY

Vitamin C was determined by analysis in the food as eaten each day by healthy nursing mothers and in 24-hour collections of their milk and urine during the first 10 days postpartum and 5-day periods at intervals later in lactation. In addition, vitamin C was determined in blood from the umbilical cord and in fasting samples of capillary blood from the mothers and infants.

The amounts of vitamin C secreted in milk paralleled the increases in milk volume, while vitamin C excretion in the urine was high after delivery and for 3 or 4 mothers decreased 50% or more by the tenth day. No relationships between volume of milk or its vitamin C content and excretion in the urine were indicated.

During the periods of secretion of mature milk, the amounts of vitamin C in the milk, in general, paralleled milk volume. For the mothers studied, whose nutritional status had been good during pregnancy, no relationship was found between vitamin C intake and secretion in milk. Excretion in urine was not related to level of intake or secretion in milk.

Extreme anatomic and physiologic variability among individuals and in the same individual at different times is emphasized by the results. The data indicate also the inconclusiveness of determinations of vitamin C levels in milk, urine or fasting blood samples as an index of vitamin C nutritional status of lactating women receiving good diets. The need for further research on the physiologic influences exerted by lactation and pregnancy on vitamin C metabolism is stressed. These include adjustment of the maternal body from pregnancy to lactation, including the glands of internal secretion, the level of requirement as opposed to maximum level of storage, and differences in requirements before pregnancy,

during gestation, and during lactation. On the basis of the data presented it seems that the Recommended Dietary Allowances provide for adequate amounts of vitamin C to meet normal requirements during lactation, provided the prenatal nutritional status of the mother has been satisfactory.

LITERATURE CITED

DAVIES, V. 1945 Human milk studies XXI. A simple technic for the manual expression of mother's milk. Am. J. Dis. Child., *70:* 148.

ESCUDERO, P., AND E. PIERANGELI 1943 Influencia de la alimentacion de la gravida sobre la riqueza mineral y vitaminica del calostro. Revista de la Asociacion Argentina de Dietologia, *1:* 85.

FARMER, C. J., AND A. F. ABT 1936 Determination of reduced ascorbic acid in small amounts of blood. Proc. Soc. Exp. Biol. Med., *34:* 146.

FAULKNER, J. M., AND F. H. L. TAYLOR 1938 Observations on the renal threshold for ascorbic acid in man. J. Clin. Invest., *17:* 69.

FOOD AND NUTRITION BOARD 1945 Recommended Dietary Allowances. National Research Council, Reprint and Circular Series 122.

HAMIL, B. M., B. MUNKS, E. Z. MOYER, M. KAUCHER AND H. H. WILLIAMS 1947 Vitamin C in the blood and urine of the newborn during the first week of life and in the cord and maternal blood. Am. J. Dis. Child. (in press.)

HARRIS, L. J., AND S. N. RAY 1935 Diagnosis of vitamin-C subnutrition by urine analysis, with a note on the antiscorbutic value of human milk. Lancet, *1:* 71.

HARRIS, L. J., S. N. RAY AND A. WARD 1933 The excretion of vitamin C in human urine and its dependence on the dietary intake. Biochem. J., *27:* 2011.

HAWLEY, E. E., J. B. FRAZER, L. L. BUTTON AND D. J. STEPHENS 1936 The effect of the administration of sodium bicarbonate and of ammonium chloride on the amount of ascorbic acid found in the urine. J. Nutrition, *12:* 215.

HESS, A. F., AND H. R. BENJAMIN 1934 Urinary excretion of vitamin C. Proc. Soc. Exp. Biol. Med., *31:* 855.

INGALLS, T. H., R. DRAPER AND H. M. TEEL 1938 Vitamin C in human pregnancy and lactation. II. Studies during lactation. Am. J. Dis. Child., *56:* 1011.

KAUCHER, M., E. Z. MOYER, A. J. RICHARDS, H. H. WILLIAMS, A. L. WERTZ AND I. G. MACY 1945 Human milk studies. XX. The diet of lactating women and the collection and preparation of food and human milk for analysis. Am. J. Dis. Child., *70:* 142.

KAUCHER, M., E. Z. MOYER, H. H. WILLIAMS AND I. G. MACY 1946 Adequacy of the diet during lactation. J. Am. Diet. Assoc., *22:* 594.

KAY, H. D. 1945 The secretion of milk. Nature, *156:* 159.

KING, C. G. 1938 The physiology of vitamin C. J. Am. Med. Assoc., *111:* 1098.

LOWRY, O. H., O. A. BESSEY, M. J. BROCK AND J. A. LOPEZ 1947 The interrelationship of dietary, serum, white blood cell, and total body ascorbic acid. J. Biol. Chem., *166:* 111.

LUND, C. J. 1945 Nutrition in pregnancy. J. Am. Med. Assoc., *128:* 344.

MACY, I. G., H. A. HUNSCHER, E. DONELSON AND B. NIMS 1930 Human milk flow. Am. J. Dis. Child., *39:* 1186.

MACY, I. G., H. H. WILLIAMS, J. P. PRATT AND B. M. HAMIL 1945 Human milk studies. XIX. Implications of breast feeding and their investigation. Am. J. Dis. Child., *70:* 135.

MUNKS, B., A. ROBINSON, H. H. WILLIAMS AND I. G. MACY 1945 Human milk studies XXV. Ascorbic acid and dehydroascorbic acid in colostrum and mature milk. Am. J. Dis. Child., *70:* 176.

PRATT, J. P., C. RODERUCK, M. CORYELL AND I. G. MACY 1946 Composition of the human placenta. III. Vitamin content. Am. J. Obstet. Gynec., *52:* 783.

RALLI, E. P., G. J. FRIEDMAN AND S. SHERRY 1939 The vitamin C requirement of man, estimated after prolonged studies of the plasma concentration and daily excretion of vitamin C in 3 adults on controlled diets. J. Clin. Invest., *18:* 705.

RODERUCK, C., M. CORYELL, H. H. WILLIAMS AND I. G. MACY 1946 Metabolism of women during the reproductive cycle. IX. The utilization of riboflavin during lactation. J. Nutrition, *32:* 267.

RODERUCK, C., H. H. WILLIAMS AND I. G. MACY 1946 Metabolism of women during the reproductive cycle. VIII. The utilization of thiamine during lactation. J. Nutrition, *32:* 249.

ROE, J. H., AND C. A. KUETHER 1943 The determination of ascorbic acid in whole blood and urine through the 2,4-dinitrophenylhydrazine derivative of dehydroascorbic acid. J. Biol. Chem., *147:* 399.

SELLEG, I., AND C. G. KING 1936 The vitamin C content of human milk and its variation with diet. J. Nutrition, *11:* 599.

SHIELDS, J. B., B. C. JOHNSON, T. S. HAMILTON AND H. H. MITCHELL 1946 The excretion of ascorbic acid and dehydroascorbic acid in sweat and urine under different environmental conditions. J. Biol. Chem., *161:* 351.

SMITH, S. L. 1938 Human requirements of vitamin C. J. Am. Med. Assoc., *111:* 1753.

TODHUNTER, E. N., AND R. C. ROBBINS 1940 Observations on the amount of ascorbic acid required to maintain tissue saturation in adults. J. Nutrition, *19:* 263.

WIDENBAUER, F., AND A. KÜHNER 1937 Ascorbinsäurestudien an stillenden Frauen. Zeitschr. *f.* Vitaminforsch., *6:* 50.

METABOLISM OF WOMEN DURING THE REPRODUCTIVE CYCLE

XII. THE EFFECT OF MULTI-VITAMIN SUPPLEMENTS ON THE VITAMIN C UTILIZATION OF NURSING MOTHERS [1]

J. P. PRATT, BERTHA MUNKS AND ICIE G. MACY
The Henry Ford Hospital; and the Research Laboratory, Children's Fund of Michigan, Detroit

(Received for publication January 30, 1947)

A preceding paper (Munks, Kaucher, Moyer, Harris and Macy, '47) reported analyses for vitamin C in the food intakes, milk, urine and blood of healthy mothers, and in the blood of their infants at various periods during lactation. The results demonstrated the wide variations in vitamin C metabolism among normal lactating women and the unreliability of levels of the vitamin in the blood and urine as an index of nutritional status with respect to the vitamin. Intakes comparable with the Recommended Allowances ('45) of the Food and Nutrition Board, from good diets of natural foods, were shown to be ample in meeting the health requirements of mothers while they were nursing their infants, although urine excretion of vitamin C was extremely variable and fasting blood levels were frequently as low as those considered to indicate scurvy in non-pregnant, non-lactating women. To extend the information concerning vitamin C metabolism in nursing mothers, the milk, urine and blood of the same subjects and the blood of their infants were analyzed after 6 to 14 days during which the women received supplemental amounts of ascorbic acid and other synthetic vitamins in

[1] The investigation represented in part by this paper was partially supported by a grant from the Nutrition Foundation, Inc.

addition to good diets. These data may be compared with the average values for the same subjects during 5-day periods of known intake immediately preceding the interval of supplement (Munks, et al., '47).

PROCEDURE

During 13 periods of 5 consecutive days for 6 healthy mothers who were successfully nursing their infants, vitamin C was determined in 24-hour collections of food, as eaten, urine and milk. Blood levels of vitamin C were determined for both the mothers and their infants. For 6 to 14 days immediately following the 5-day periods the mothers received 750 mg of ascorbic acid, 50,000 U.S.P. units of vitamin A, 12 mg of thiamine chloride, 15 mg of riboflavin and 120 mg of niacin.[2] To distribute the intake of the vitamins over the day (Ralli, Friedman and Sherry, '39) the supplement of the water-soluble vitamins was given in 3 equal capsules, 1 with each meal. For the last day of the supplemented diet all milk and urine was collected and a fasting blood sample from the mother, after expression of the milk at 6 A.M., and a blood sample from the infant were obtained at the end of the 24 hours.

Analyses of food, milk and urine for vitamin C were made by the Roe and Keuther method ('43) and of the blood by the method outlined by Farmer and Abt ('36). Details of the selection of subjects (Macy et al., '45), diets (Kaucher, Moyer, Williams and Macy, '46), the methods of manually expressing the milk (Davies, '45) and procedures of collecting the food composites, milk and urine (Kaucher, Moyer, Richards, Williams, Wertz and Macy, '45) are given elsewhere. The concentration of vitamin C in colostrum and mature breast milk (Munks et al., '45) and the nutritional state of the nursing mothers with respect to vitamin C (Munks and coworkers, '47) have been recorded.

[2] The amounts of the vitamins given as supplements were less than therapeutic dosages, defined as 10 times the daily requirements, respectively.

RESULTS

Table 1 presents the average daily values for vitamin C in the food, breast milk and urine during the 5-day control periods and the results of analyses of 24-hour samples collected on the last day of supplementation of the diet with 750 mg of ascorbic acid.

The concentration of vitamin C in the milk was in some instances more than doubled and was lowered only for 1 study (V.G.) although several of the daily values were less after supplement, owing to the smaller volumes of milk secreted. However, the average concentration was increased only 2 mg, from 6 to 8 mg per 100 ml. The largest amount of vitamin C secreted per day after supplementation with 750 mg per day was 76 mg by V.G. during her third month of lactation. During the control studies the mothers, weighing from 55 to 73 kg, were eating daily diets which by analysis contained averages of 102 to 228 mg vitamin C, although by calculation from tables of food composition the vitamin C contents of the diets ranged from 132 to 169 mg per day.

Although there may be an upper limit to the concentration of vitamin C an individual woman can secrete in her milk, the limit does not seem to be the same for every individual. The highest value, 12.4 mg per 100 ml, was found in the milk of a woman (V.S.) in whom lactation was almost terminated. However, before the supplement was taken this woman was secreting milk containing 9.5 mg per 100 ml. Another woman secreted 10.1 and 10.5 mg per 100 ml, respectively, after supplementation in the third and sixth months of lactation, although during the control period her milk contained only 4.4 and 5.3 mg per 100 ml, respectively. After 4 supplementation periods at least 2 months apart, values for a third woman were changed from 5.4, 5.0, 7.0 and 7.5 mg to 7.5, 7.1, 3.8 and 7.5 mg per 100 ml, respectively.

The concentrations in urine following the supplement were as much as 140 times (V.G.) those of the control period. The total daily excretions after supplement ranged from 504 to 680 mg greater than the averages for the 5 days of the control

TABLE 1

Vitamin C in milk and urine before and after supplement.[1]

SUBJECT	CONTROL						AFTER SUPPLEMENT				
	Interval postpartum	Intake	Milk		Urine		Interval postpartum	Milk		Urine	
	days	*mg*	*mg/100 ml*	*mg/day*	*mg/100 ml*	*mg/day*	*days*	*mg/100 ml*	*mg/day*	*mg/100 ml*	*mg/day*
V.G.	78–82	128	5.4	46	0.4	5	92	7.5	76	56	531
	161–165	105	5.0	45	0.6	6	175	7.1	62	..	...
	239–243	102	7.0	48	5.6	44	253	3.8	27	75	689
	302–306	143	7.5	29	3.2	34	316	7.5	24	66	632
V.K.	95–99	102	6.5	42	0.3	9	109	8.9	62	33	613
	144–148	145	8.0	26	0.8	12	158	9.8	32	60	516
V.L.	68–72	162	6.3	50	0.4	10	82	8.2	64	38	665
	152–156	156	6.4	43	2.2	37	170	6.8	35	47	717
J.M.	75–79	138	4.4	31	0.2	5	89	10.1	61	18	612
	173–177	131	5.3	14	0.2	4	188	10.5	10	29	529
B.S.	204–208	130	4.1	37	0.5	6	218	5.8	57	33	529
	259–263	115	4.9	33	.06	7	273	6.8	44	60	655
V.S.[2]	70–74	228	9.5	29	5.0	92	80	12.4	16	22	307

[1] 750 mg ascorbic acid per day.

[2] Owing to rapid failure of lactation the period of supplement for V.S. was only 5 days.

period with 1 exception. V.S. during the control period secreted milk with the highest concentration of the vitamin and excreted more than double the amount per day excreted by any other woman, yet after receiving 750 mg per day in addition to her diet for 5 days she excreted only 215 mg more in 24 hours than her average excretion per day during 5 days preceding supplement.

TABLE 2

Vitamin C in blood serum of mother and infant before and after supplement. (mg/100 ml.)

SUBJECT	CONTROL PERIOD			AFTER SUPPLEMENT		
	Interval postpartum	Mother [1]	Child	Interval postpartum	Mother	Child
	days			*days*		
V.G.	78	0.30	0.50	92	0.90	0.50
	161	0.21	1.72	175	1.04	1.40
	239	0.61 (0.96)	0.81	253	2.15	0.57
	302	0.16 (0.60)	1.07	316	1.31	1.19
V.K.	95	0.80	3.00	109	1.30	0.66
	144	0.73	0.92	158	1.34	0.73
V.L.	68	0.26	1.46	83	1.55	1.63
	152	0.80 (0.80)	1.43	170	1.39	1.59
J.M.	75	0.17	0.74	90	1.45	1.20
	172	0.20 (0.55)	0.64	188	1.19	0.84
B.S.	85	0.20	1.00	99	1.20	1.20
	204	0.16	0.79	218	0.94	0.90
	259	0.38	0.57	273	1.36	0.98
M.S.	58	0.04	0.63	72	1.12	1.21
V.S.	70	0.13 (0.36)	0.82	80	1.46	0.42

[1] Values in parentheses were obtained at the end of the 5-day control period. All other values represent the values on the first day of control period.

The estimations of vitamin C in the fasting samples of blood of the mothers and infants during the control period and following supplementation of the diet are given in table 2. Of the 15 values for blood obtained from the mothers on the first days of control periods, only 4 were above 0.6 mg per 100 ml, the amount regarded as comparable with health

(Smith, '38; Goldsmith and Ellinger, '39). The others were from 0.04 to 0.38 mg per 100 ml, an amount generally thought to indicate deficiency of vitamin C in the tissues. These results agree with those of Ingalls, Draper and Teel ('38) who found 0.3 mg per 100 ml on the fourteenth day postpartum. In 5 instances vitamin C was determined also in samples obtained after the mothers had received for 5 days the diet supplied during the control period. The blood level remained unchanged at 0.8 mg for 1 subject (V.L.) but the other 4 values showed increases of 0.35, 0.44, 0.35, and 0.23 mg.

After increasing the daily intake of vitamin C 4 to 8 times, the blood serum values of the women increased to within the range of saturation for non-pregnant non-lactating women (Storvick and Hauck, '42; Goldsmith and Ellinger, '39; Faulkner and Taylor, '38; Todhunter and Robbins, '40; Fincke and Landquist, '42), ranging from 0.9 to 2.2 mg per 100 ml of blood serum. The increases in concentration, with 1 exception, ranged from 1.6 to 11 times the values obtained the first day of the control period. The blood of M.S., was extremely low, 0.04 mg, at the start of the control period and increased to 1.12 mg following supplementation of her diet. Values after supplement were only 2 to 4 times greater than the determinations made following 5 days of eating the diet given during the control period.

DISCUSSION

Traversaro and Quesada ('38) have stated that the upper limit of vitamin C concentration in the blood and milk can be reached with food, or with crystalline vitamin C in the case of deficiency. Selleg and King ('36) observed that mothers with high initial vitamin C concentrations in milk showed relatively small increases when vitamin C supplements were given. It has been suggested (Traversaro and Quesada, '38; Baumann, '37) that there is an upper limit to the vitamin C secretion by the mammary gland, probably averaging between 7 and 8 mg per 100 ml. The data obtained in the present study support the belief in an upper limit of vitamin C concentra-

tion in human milk but emphasize the variability of that limit in different women.

Ingalls, Draper and Teel ('38) gave 300 and 600 mg vitamin C to nursing women during the first 2 weeks postpartum. After the first week the group receiving 600 mg of vitamin C per day had no greater concentration of vitamin C in their blood and milk than the group receiving 300 mg. They concluded that both groups had reached saturation at the end of the first week and that the excess vitamin was probably excreted in the urine. The ascorbic acid of the blood after 1 and 2 weeks rose to 1.38 and 1.44 mg per 100 ml in the supplemented groups. The milk values ranged from 5.0 to 9.5 and averaged 7.2 and 7.4 mg per 100 ml at the end of the first and second weeks, respectively. Selleg and King ('36) supplemented the diets of 2 groups of women with orange juice, equivalent to 210 and 430 mg of vitamin C per day during the first 10 days postpartum. The vitamin C concentration in the milk after supplementation rose to 7.3 and 8.1 mg per 100 ml on the tenth day. It would appear that the supplements given in these 2 studies served merely to bring the subjects' dietary intakes to an adequate level or to offset earlier poor nutritional state, for the values attained with the supplements compare with data presented from this laboratory (Munks, Robinson, Williams and Macy, '45) for the concentration of vitamin C in milk secreted during the first 10 days postpartum by healthy mothers receiving vitamin C only from a good diet of natural foods.

Widenbauer and Kühner ('37) determined for 6 mothers, 1 week to 16 months postpartum, the ascorbic acid in urine and milk per day during a preperiod and during a period of 20 to 27 days of oral administration of 400 to 500 mg of ascorbic acid per day. The subjects had very poor dietary histories and the study diets contained, besides potatoes and vegetables, small amounts of salad, but no fruit. When vitamin C excretion in urine rose a little, less vitamin C was given until excretion rose abruptly. The amount was then reduced until an approximate value for daily requirement

was reached. The ascorbic acid content of the breast milk was 0.5 to 2.2 mg per 100 ml before and 3.8 to 7.5 mg following supplementation. They comment that the values in the literature (4 to 7 mg per 100 ml) must have been obtained with milk from women who ate good diets with respect to vitamin C and conclude that their 6 women were vitamin C deficient to a greater or lesser extent as demonstrated by the saturation deficit. The daily ascorbic acid secretions in the milk before saturation were 5, 5, 4, 14, 13, and after supplementation were 10 mg and 60, 20, 18, 68, 78, 34 mg, respectively, increases to 3 to 11 times the earlier values, whereas the values reported in the present paper were only doubled. The daily excretions of the vitamin after supplement reported by Widenbauer and Kühner are within the range of the values in table 1 and indicate that the magnitude of increases in excretion of vitamin C supplements is dependent on the diets which the mothers have received for some time.

Widenbauer and Kühner concluded that the nursing mother requires 80 to 100 mg vitamin C per day in order to secrete 40 to 50 mg for the infant. From the data presented in this and the preceding paper it would seem necessary to supply the mother with considerably larger amounts, especially if based on values calculated from the literature, to assure the infant 40 to 50 mg per day. However, whether infants actually require that much vitamin C is not known. To eliminate the possibility of inadequate intakes of this important vitamin, common pediatric practice is the early addition of orange juice to even the breast-fed baby's diet.

It has been observed that there is a relatively slow and limited rise in the antiscorbutic value of breast milk when patients receive a special supplement of fruit juice equivalent to 210 or 430 mg of ascorbic acid per day. Sel'eg and King ('36) considered this noteworthy in that it provided an indication of a maximum and approximately optimum level of secretion, above which an excessive dietary intake results chiefly in a rapid urinary excretion without disturbing the lactation level. Other investigators (Chu and Sung, '36) found that a

sudden increase in the urinary excretion of vitamin did not occur until a large dose of vitamin had been given for 3 to 6 days, showing that the storage of vitamin C in the tissues of the mothers was very low at the beginning of the experiment. Once the peak was reached, the daily urinary output of vitamin C tended to be fairly constant, amounting to about 60% of the intake. They observed that excretion dropped abruptly with the discontinuance of the added vitamin.

Similar to the observation of Selleg and King, in a study of underfed mothers Chu and Sung ('36) observed that with an increase or decrease of vitamin C supply in the diet there was a corresponding fluctuation in the vitamin C content of milk, but the change was very slow and steady, being entirely different from the type of response in the urinary excretion. They suggest that "since milk is a product of secretion rather than excretion, it behaves like body tissue in this respect." After it had reached a saturation level, which was around 8 mg per 100 ml, the concentration of vitamin C in the milk remained high for about 10 days, even after the extra supply of the vitamin was discontinued.

The preceding paper emphasized the impossibility, at present, of distinguishing vitamin C storage from requirement for the substance. The same paper demonstrated that vitamin C in fasting blood samples could not be used as a criterion of the health of a nursing mother, the adequacy of her diet, or the vitamin C content of her milk and that there is a wide range of normal variation among healthy women. In the present study administration of 750 mg of ascorbic acid per day to healthy mothers eating good diets and successfully nursing their babies produced increases in the concentration of the vitamin in their milk and in fasting samples of their blood. However, these increases accounted for only a small fraction of the increases in intake, the greater portion of which was excreted in urine. During the control period the infants' blood levels (table 2) were much higher than their mothers', ranging from 0.50 to 3.00 mg per 100 ml. After the period of supplement the infants' blood contained 0.42

to 1.63 mg of vitamin C per 100 ml. In only 9 of 15 instances did the blood levels increase following ingestion of their mothers' milk with increased concentration. Without any evidence that the increased levels in the mothers' blood were beneficial to them, or that the increased concentrations in milk were helpful to their infants, the conclusion of the preceding paper seems justified: ". . . it seems that the Recommended Dietary Allowances ('45) of the Food and Nutrition Board of the National Research Council provide for adequate amounts of vitamin C to meet normal requirements during lactation and prevent depletion of body stores, provided during pregnancy the nutritional status of the mother has been adequate with respect to the vitamin."

SUMMARY

Values obtained by analysis for vitamin C in the milk, blood and urine of healthy mothers and in the blood of their breast-fed infants following administration of 750 mg of ascorbic acid per day for 6 to 14 days, have been compared with corresponding data for the same women and their infants during 5-day intervals immediately preceding the supplement period when analyses of the mothers' diets showed average intakes to be 102 to 228 mg of vitamin C per day.

Although the average levels of vitamin C in the breast milk and in fasting blood samples were higher following supplementation of the diet, the major portion of the ascorbic acid given was represented by increases in urinary excretion. The data suggest that the amount of vitamin C recommended by the Food and Nutrition Board for lactating women is sufficient if supplied by natural foods in a diet adequate with respect to other dietary essentials and if during pregnancy the nutritional status of the mother has been satisfactory.

LITERATURE CITED

BAUMANN, T. 1937 Untersuchungen über den C-Vitaminstoffwechsel bei laktierenden Frauen und über den Grad der physiologischen und pathologischen C-Vitamin sättigung des menschlichen Organismus. Jahrb. f. Kinderh., *150:* 193.

CHU, F. T., AND C. SUNG 1936 Effect of vitamin C administration on vitamin C of milk and urine of lactating mothers. Proc. Soc. Exp. Biol. Med., *35:* 171.

DAVIES, V. 1945 Human milk studies XXI. A simple technic for the manual expression of mothers' milk. Am. J. Dis. Child., *70:* 148.

FARMER, C. J., AND A. F. ABT 1936 Determination of reduced ascorbic acid in small amounts of blood. Proc. Soc. Exp. Biol. Med., *34:* 146.

FAULKNER, J. M., AND F. H. L. TAYLOR 1938 Observations on the renal threshold for ascorbic acid in man. J. Clin. Invest., *17:* 69.

FINCKE, M. L., AND V. L. LANDQUIST 1942 The daily intake of ascorbic acid required to maintain adequate and optimal levels of this vitamin in blood plasma. J. Nutrition, *23:* 483.

FOOD AND NUTRITION BOARD 1945 Recommended Dietary Allowances. National Research Council Reprint and Circular Series No. 122.

GOLDSMITH, G. A., AND G. F. ELLINGER 1939 Ascorbic acid in blood and urine after oral administration of a test dose of vitamin C. A saturation test. Arch. Int. Med., *63:* 531.

INGALLS, T. H., R. DRAPER AND H. M. TEEL 1938 Vitamin C in human pregnancy and lactation. II. Studies during lactation. Am. J. Dis. Child., *56:* 1011.

KAUCHER, M., E. Z. MOYER, A. J. RICHARDS, H. H. WILLIAMS, A. L. WERTZ AND I. G. MACY 1945 Human milk studies. XX. The diet of lactating women and the collection and preparation of food and human milk for analysis. Am. J. Dis. Child., *70:* 142.

KAUCHER, M., E. Z. MOYER, H. H. WILLIAMS AND I. G. MACY 1946 Adequacy of the diet during lactation. J. Am. Diet. Assoc., *22:* 594.

MACY, I. G., H. H. WILLIAMS, J. P. PRATT AND B. M. HAMIL 1945 Human milk studies. XIX. Implications of breast feeding and their investigation. Am. J. Dis. Child., *70:* 135.

MUNKS, B., M. KAUCHER, E. Z. MOYER, M. E. HARRIS AND I. G. MACY 1947 Metabolism of women during the reproductive cycle. XI. Vitamin C in diets, breast milk, blood and urine of nursing mothers. J. Nutrition, *33:* 601.

MUNKS, B., A. ROBINSON, H. H. WILLIAMS AND I. G. MACY 1945 Human milk studies. XXV. Ascorbic acid and dehydroascorbic acid in colostrum and mature human milk. Am. J. Dis. Child., *70:* 176.

RALLI, E. P., G. J. FRIEDMAN AND S. SHERRY 1939 The vitamin C requirement of man, estimated after prolonged studies of the plasma concentration and daily excretion of vitamin C in 3 adults on controlled diets. J. Clin. Invest., *18:* 705.

ROE, J. H., AND C. A. KUETHER 1943 The determination of ascorbic acid in whole blood and urine through the 2,4 dinitrophenyl-hydrazine derivative of dehydroascorbic acid. J. Biol. Chem., *147:* 399.

SELLEG, I., AND C. G. KING 1936 The vitamin C content of human milk and its variation with diet. J. Nutrition, *11:* 599.

SMITH, S. L. 1938 Human requirements of vitamin C. J. Am. Med. Assoc., *111:* 1753.

STORVICK, C. A., AND H. M. HAUCK 1942 Effect of controlled ascorbic acid ingestion upon urinary excretion and plasma concentration of ascorbic acid in normal adults. J. Nutrition, *23:* 111.

TODHUNTER, E. N., AND R. C. ROBBINS 1940 Observations on the amount of ascorbic acid required to maintain tissue saturation in normal adults. J. Nutrition, *19:* 263.

TRAVERSARO, J. C., AND R. QUESADA 1938 Vitamina C en la leche de mujer. Rev. Assoc. Bioquim. Argentina, *3:* 7.

WIDENBAUER, F., AND A. KÜHNER 1937 Ascorbinsäurestudien an stillenden Frauen. Zeitschr. f. Vitaminfors., *6:* 50.

BLOOD LEVELS AND ABSORPTION OF VITAMIN A IN CHILDREN WITH KERATOSIS FOLLICULARIS [1]

R. F. KRAUSE AND HAROLD B. PIERCE
WITH THE TECHNICAL ASSISTANCE OF
RUTH LAW AND HATTIE KAPLAN
Department of Biochemistry, College of Medicine, University of Vermont, Burlington

TWO FIGURES

(Received for publication February 17, 1947)

It has been postulated by Peck et al. ('43) that keratosis follicularis is a deficiency syndrome which may be caused by either a hereditary or an acquired weakness in absorption of vitamin A or in conversion of provitamin A to vitamin A. In 10 cases of this disease he reports 8 with the vitamin A content of the blood below "normal limits," and 2 at the lower limits of "normal." Carelton and Steven ('43) and Cornbleet et al. ('44) report a total of 5 cases of this disease with no evidence of vitamin A deficiency in the blood.

In view of these different findings it was believed worthwhile to investigate, first, if individuals with folliculosis do have abnormally low vitamin A and carotene serum levels and secondly, if these individuals exhibit a weakness in the absorption of vitamin A.

METHODS AND PROCEDURES

Children were selected for study since Pierce et al. ('45) found a high incidence of folliculosis among Burlington chil-

[1] The work was supported in part by grants from Merck and Company, Inc., Milbank Memorial Fund and National Vitamin Foundation, Inc.

dren. Children from Burlington Public and Parochial schools and from St. Joseph's Orphanage were examined for the presence of folliculosis.[2] Two hundred and fifteen were chosen for the folliculosis group and 179 for the non-folliculosis group. Finger-tip blood was taken from each child at the time of the physical examination for the determination of vitamin A and carotene by the methods of Bessey et al. ('46b).

Vitamin A absorption studies were made on 36 Orphanage children between the ages of 8 and 15 years. Nineteen of these children had folliculosis and were used as a "test group" and 17 without folliculosis were used as a "control group." Vitamin A capsules[3] containing 50,000 I.U. were given to 12 "test" and 12 "control" children and 25,000 I.U. to 7 "test" and 5 "control" children. Finger-tip blood was taken prior to the vitamin administration and 4, 5, 6, 8 and 24 hours thereafter, for vitamin A and carotene determinations.

No effort was made to control the diet of these children since it has been shown that ordinary mixed meals have little or no effect upon the concentration of either vitamin A or carotene in serum (Kimble, '39). Then too, if any change did occur in serum levels due to food ingested it would be relatively constant for all children since they were institutionalized and eating the same food.

RESULTS

Table 1 summarizes the data obtained from the determination of serum vitamin A and carotene values of children with and without folliculosis. The average vitamin A concentrations for both groups of children were the same, namely 38 μg %, whereas the average carotene values were significantly lower in the group with folliculosis.

[2] The extremities were examined for the presence of keratosis follicularis by Drs. John H. Browe, Research Associate in Medicine; C. A. Newhall, Assoc. Prof. Anatomy; T. H. Harwood, Asst. Prof. Medicine and P. D. Clark, Asst. Prof. Pediatrics, Medical College, University of Vermont.

[3] The vitamin A used in this study was supplied by Eli Lilly and Company, Indianapolis, Ind.

TABLE 1

Serum concentration of vitamin A and carotene of children with and without folliculosis.

	CHILDREN WITH FOLLICULOSIS		CHILDREN WITHOUT FOLLICULOSIS	
	Vitamin A	Carotene	Vitamin A	Carotene
Number of children	215	215	179	179
Micrograms per cent	38	101	38	116
Standard deviations	± 9.8	± 16.5	± 10.3	± 45.7

TABLE 2

Vitamin A serum levels of test and control children after oral administration of 50,000 I.U. of vitamin A.

SUBJECT	BASAL	HOUR AFTER VITAMIN A ADMINISTRATION				
		4	5	6	8	24
		μg % vitamin A in serum				
			Control Group			
1	35	141	187	88	58	42
2	32	51	78	81	44	47
3	27	98	128	69	75	41
4	27	61	85	53	47	36
5	26	62	129	138	..	40
6	28	33	40	51	54	39
7	30	43	194	88	74	38
8	26	90	196	111	46	28
9	19	37	159	120	41	34
10	19	29	84	100	47	31
11	36	81	114	95	58	29
12	27	81	91	50	39	21
Average	27.6	67.2	123.8	87.0	52.9	35.5
St. dev.	± 5.4	± 31.2	± 49.0	± 26.9	± 11.6	± 6.9
			Test Group			
13	29	84	183	122	67	36
14	30	54	80	56	86	48
15	34	67	212	122	74	66
16	27	21	48	30	28	37
17	46	91	118	75	60	71
18	31	26	88	86	80	40
19	26	81	171	126	86	26
20	18	46	126	107	98	25
21	26	43	171	156	75	38
22	23	12	39	45	47	32
23	27	37	152	97	40	35
24	19	48	100	121	67	34
Average	28.0	50.8	123.9	95.5	67.3	40.6
St. dev.	± 6.9	± 10.9	± 20.8	± 13.1	± 6.7	± 4.4

TABLE 3

Vitamin A serum levels of test and control children after oral administration of 25,000 I.U. of vitamin A.

SUBJECT	BASAL	HOUR AFTER VITAMIN A ADMINISTRATION				
		4	5	6	8	24
		μg % vitamin A in serum				
		Control Group				
25	20	103	41	49	22	18
26	28	77	83	78	71	33
27	15	86	105	118	51	26
28	28	37	64	55	..	24
29	37	39	48	41	39	38
Average	26	68	68	68	45	28
St. dev.	± 7.6	± 26.2	± 23.4	± 27.8	± 17.9	± 7.0
		Test Group				
30	33	50	58	..	46	32
31	24	13	22	34	38	30
32	15	23	66	44	34	15
33	29	59	57	50	35	31
34	22	88	87	65	32	27
35	23	31	90	46	26	21
36	21	26	97	150	58	22
Average	24	41	68	65	38	25
St. dev.	± 5.4	± 24.0	± 20.8	± 39.2	± 9.8	± 5.8

In the absorption study, the basal vitamin A levels and those for 4, 5, 6, 8 and 24 hours after the administration of vitamin A are presented in tables 2 and 3. The average rise in serum vitamin A and the ensuing average drop for each interval of time after the ingestion are noted, for both the "test" and "control" groups, in figures 1 and 2, and the resulting curves are designated as average "tolerance" curves.

In all but 2 cases the maximum rise in serum concentration was reached on either the fifth or sixth hour after ingestion.

Fig. 1 The average "tolerance" curves for 12 children with folliculosis (Test group) and 12 children without folliculosis (Control group) after the ingestion of 50,000 I.U. of vitamin A.

Fig. 2 The average "tolerance" curves for 5 children without folliculosis (Control group) and 7 children with folliculosis (Test group) after the ingestion of 25,000 I.U. of vitamin A.

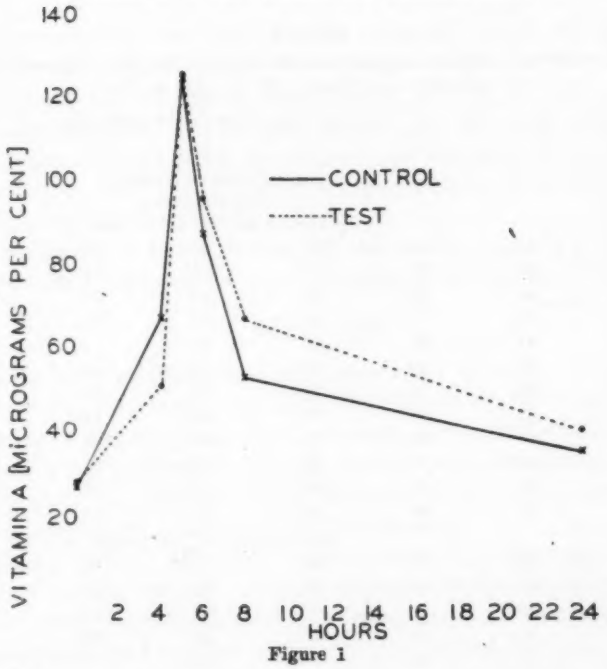


Figure 1

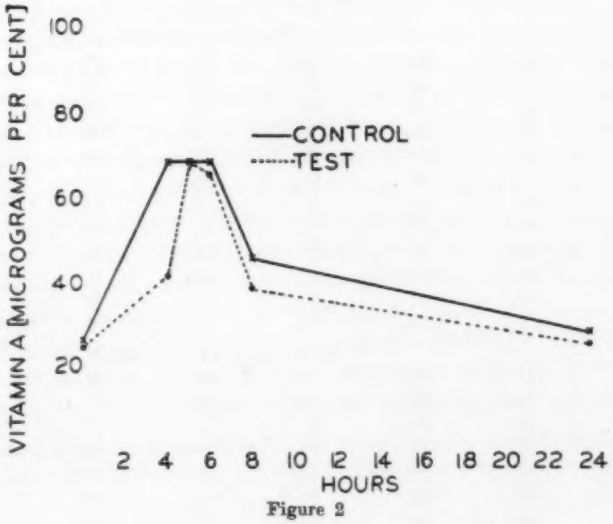


Figure 2

TABLE 4

Serum carotene levels of test and control children after oral administration of 50,000 and 25,000 I.U. of vitamin A.

SUBJECT	BASAL	HOUR AFTER VITAMIN A ADMINISTRATION				
		4	5	6	8	24
			μg % vitamin A in serum			
			Control Group			
			After 50,000 I.U. of vitamin A			
1	74	69	78	67	68	72
2	79	79	75	76	76	76
3	91	89	96	92	95	89
4	61	57	63	61	61	64
5	64	61	61	65	..	68
6	67	65	66	62	67	62
7	90	86	85	84	89	86
8	82	78	75	75	74	79
9	109	106	95	100	104	107
10	84	88	79	80	84	84
11	120	124	112	110	113	110
12	88	88	83	80	85	84
			After 25,000 I.U. of vitamin A			
25	75	75	70	73	69	70
26	85	87	84	85	83	80
27	50	55	51	55	53	51
28	54	54	51	50	53	50
29	32	32	30	29	30	33
			Test Group			
			After 50,000 I.U. of vitamin A			
13	62	64	59	58	62	68
14	77	75	74	74	80	74
15	80	79	85	83	79	83
16	87	86	86	86	90	86
17	80	78	78	80	78	82
18	55	56	60	55	52	54
19	56	54	54	55	54	56
20	94	100	94	92	94	94
21	60	62	62	61	58	59
22	62	59	54	58	60	59
23	75	71	71	70	70	73
24	77	76	73	73	75	77
			After 25,000 I.U. of vitamin A			
30	31	32	28	..	31	29
31	54	60	54	57	54	58
32	28	29	27	27	28	31
33	57	56	54	54	60	55
34	66	59	58	57	57	55
35	79	85	81	77	80	81
36	43	47	44	45	46	44

This maximum rise was, on the average, about 4½ and 2½ times higher than the average basal values for the 50,000 and 25,000 I.U. dosages, respectively. At the end of 8 hours the concentration was reduced to one-half its peak value and by 24 hours was back to normal for the groups receiving 25,000 I.U. but was still slightly elevated for the group receiving the higher dosage.

The basal carotene levels and those for 4, 5, 6, 8 and 24 hours after administration of vitamin A are shown in table 4.

DISCUSSION

It is evident from the data presented in table 1 that the children in this study who have folliculosis do not have lower vitamin A serum levels than "normal" children. It is interesting to note, however, that the serum carotene concentration was lower in children with folliculosis. No information is available for an explanation of this finding.

Bessey ('46a) has stated that, from his experiences, the normal serum vitamin A and carotene levels range between 30–70 μg % and 100–300 μg %, respectively. In this study the average serum vitamin A and carotene values for both groups of children lie within the lower limits of these ranges.

It may be noted in tables 2 and 3 that the concentration of vitamin A in the serum at any one time after the ingestion of this vitamin is variable from child to child and that the rate and magnitude of these changes are not necessarily related to the basal vitamin A levels[4] or to the presence or absence of folliculosis. The average "tolerance" curves for children with and without folliculosis are not significantly different. In view of these findings it is concluded that the children in this study who had folliculosis did not exhibit a weakness in absorption of vitamin A.

The serum carotene levels were not affected by the dosages of vitamin A ingested and remained essentially constant throughout the 24-hour absorption study period (see table 4).

[4] Steigmann and Popper ('44) have reported similar findings in their study entitled "The influence of large doses of vitamin A upon the plasma vitamin A level."

These data present additional confirmatory evidence for the fact that large doses of vitamin A do not alter the concentration of carotene in serum; and that the reaction, carotene → vitamin A appears to be an irreversible one (Peters and Van Slyke, '46).

SUMMARY

1. In a survey of 394 children no significant difference was found between the serum vitamin A levels of children with and without folliculosis.

2. The serum carotene values were lower in children with folliculosis.

3. The children of both groups are equally capable of absorbing vitamin A as indicated by their serum levels after the administration of 50,000 and 25,000 I.U. of vitamin A.

ACKNOWLEDGMENT

The authors wish to express their thanks for the assistance and cooperation given this study by the personnel of St. Joseph's Orphanage and Burlington's Public and Parochial Schools.

LITERATURE CITED

BESSEY, O. A. 1946 Personal communication.

BESSEY, O. A., O. H. LOWRY, M. J. BROCK AND J. A. LOPEZ 1946b The determination of vitamin A and carotene in small quantities of blood serum. J. Biol. Chem., *166:* 177.

CARLETON, A., AND D. STEVEN 1943 Keratosis Follicularis. Arch. Derm. and Syph., *48:* 143.

CORNBLEET, T., H. POPPER AND F. STEIGMANN 1944 Blood vitamin A and cutaneous diseases. Arch. Derm. and Syph., *49:* 103.

KIMBLE, M. S. 1939 The photocolorimetric determination of vitamin A and carotene in human plasma. J. Lab. Clin. Med., *24:* 1055.

PECK, S. M., A. W. GLICK, H. H. SOBOTKA AND L. CHARGIN 1943 Vitamin A studies in cases of Keratosis Follicularis (Darier's Disease). Arch. Derm. and Syph., *48:* 17.

PETERS, J. P., AND D. D. VAN SLYKE 1946 Quantitative Clinical Chemistry, *1:* 611.

PIERCE, H. B., P. F. FENTON, W. WILKINS, M. E. NEWLAND AND P. S. BUDGE 1945 Nutritional defects among children in Vermont. New Eng. J. Med., *233:* 612.

STEIGMANN, F., AND H. POPPER 1944 The influence of large doses of vitamin A upon the plasma vitamin A level. Am. J. Med. Sci., *207:* 468.

THE EFFECT OF FAT LEVEL OF THE DIET ON GENERAL NUTRITION

III. WEIGHT LOSS, MORTALITY AND RECOVERY IN YOUNG ADULT RATS MAINTAINED ON RESTRICTED CALORIES [1]

BRADLEY T. SCHEER, JOHN F. CODIE AND H. J. DEUEL, JR.
Department of Biochemistry and Nutrition, University of Southern California, School of Medicine, Los Angeles

(Received for publication January 2, 1947)

Previous papers have described the effects of feeding diets varying in fat content ad libitum, and in restricted amounts, to weanling rats (Deuel et al., '47; Scheer et al., '47a). In the experiments to be reported here, rats were maintained on a stock diet for 19 weeks after weaning, and then transferred to the experimental diets, fed in restricted amounts. Measurements of body weight and physical capacity were made during the period of caloric restriction and a subsequent recovery period of ad libitum feeding; reproductive performance was also tested in the recovery period. The methods used have been described earlier (Deuel et al., '47; Scheer et al., '47b). During the present work the restricted food portions were fed daily, instead of every second day, as in the earlier experiments. Three hundred rats were divided into groups at weaning and kept in large cages in groups of 4 for 19 weeks. At the end of

[1] The subject matter of this paper has been undertaken in cooperation with the Committee on Food Research of the Quartermaster Food and Container Institute for the Armed Forces. The opinions or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the War Department.

We are indebted to Merck and Co., Rahway, N. J., for the synthetic B vitamins; to the Winthrop Chemical Co., Albany, N. Y., for the crystalline vitamin D_3 and to Lederle Laboratories, Inc., Pearl River, N. Y., for the folic acid.

this time, they were transferred to smaller individual cages and fed the experimental diets in restricted amounts.

RESULTS

Weight loss

On the basis of a preliminary experiment, in which young adult rats were maintained on the stock diet at various levels of caloric intake for 6 weeks, it was calculated that an intake of 24 cal. per day would result in a weight loss of 50% in 12 weeks. After 8 weeks on the experimental regime, the weights of the animals receiving the diets with various amounts of fat at a level of 24 cal. per day were greater than had been predicted (table 1); we accordingly reduced the caloric intake to 12 cal. per day. After a total experimental period of 12 weeks, the males had lost 40 to 50% of their initial weight, while the females had lost 30 to 40%.

Table 1 presents the mean weights with their standard errors at intervals of 4 weeks during the restricted period. It is apparent that the greatest weight loss was observed on diet 60b, containing no fat but with 1% methyl linolate, while the smallest losses were observed on diet 63, containing 20% fat, and the stock diet, containing 14% fat. Those differences which might have occurred by chance less than once in 100 samples (difference/standard error > 2.5) are also tabulated in table 1. The results presented appear to justify the conclusion that loss of weight under conditions of severe caloric restriction is greater when the diet contains 10% of fat or less than when it provides 15 to 40% of fat. However, there is no evident advantage of the 40% level over 20%. The apparent superiority of the stock diet during the last weeks of the experiment resulted from a failure of appetite in the other groups, as will be noted later.

Mortality

In earlier work, we noted that mortality among weanling rats fed restricted calories was highest on diet 61 (5% fat) and least on the stock diet (14%) and diet 64 (40%). A very

TABLE 1

Weights of young adult rats fed experimental diets in amounts to provide 24 cal. per day for 8 weeks, followed by 12 cal. per day for 4 weeks.

DIET NO.	FAT CONTENT IN %	BODY WEIGHT IN GM [1]				M.D. : S.E.M.D.[2]	
		Start	4 wks.	8 wks.	12 wks.	8-wk. period	12-wk. period
				Male rats			
60B	0 [3]	269.5 ± 5.4	220.7 ± 1.5	200.1 ± 3.8	126.5 ± 4.5	62, 3.0; 63, 3.9; S., 3.1.	63, 3.4; 64, 3.2.
61	5	264.9 ± 2.0	223.2 ± 3.7	211.8 ± 3.9	128.0 ± 4.6		63, 3.1; 64, 2.9.
62	10	274.9 ± 5.7	235.2 ± 3.6	218.4 ± 4.7	134.6 ± 3.9		63, 2.5.
63	20	267.4 ± 7.1	225.7 ± 4.8	216.7 ± 2.0	147.9 ± 4.4		
64	40	263.1 ± 6.2	215.2 ± 5.2	201.3 ± 4.6	142.6 ± 12.1	62, 2.6; 63, 3.1.	
S [4]	14	268.4 ± 4.5	224.6 ± 2.9	213.7 ± 2.3	145.6 ± 6.5		
				Female rats			
60B	0 [3]	183.4 ± 3.2	163.3 ± 2.6 [5]	164.1 ± 1.4	116.2 ± 5.5	61, 3.5; 62, 4.0; 63, 5.2; S., 3.8.	S., 3.1.
61	5	189.2 ± 5.7	168.9 ± 1.2	171.6 ± 1.9	105.0 ± 2.5		63, 5.0; S., 8.5.
62	10	188.7 ± 3.1	176.0 ± 2.0	180.3 ± 2.0	113.8 ± 3.5	60B, 4.0. [3]	S., 4.9.
63	20	182.9 ± 3.6	169.3 ± 2.4	177.7 ± 2.2	124.7 ± 3.1		S., 2.5
64	40	188.6 ± 3.4	167.3 ± 2.6	169.7 ± 2.3	121.5 ± 7.6		
S [4]	14	189.8 ± 8.9	171.6 ± 3.1	174.1 ± 2.2	134.6 ± 2.4		

[1] Including the standard error of the mean calculated as follows $= \sqrt{\Sigma d^2/n}/\sqrt{n}$ where "d" is the deviation from the mean and "n" is the number of observations.

[2] Ratio of mean difference to standard error of mean difference. When this is 2.5, it indicates that the chances that such a difference might arise by chance are no more than 1 to 100.

[3] Containing 1.0% methyl linolate.

[4] Stock diet.

[5] Ratio M.D. : S.E.M.D. for 4-week period.

similar distribution of mortality among the groups was noted in this study (table 2). In the present experiment, death was often preceded by appearance of bloody incrustations on the coat, and lack of appetite. The bloody coats were especially evident on the abdomen and forelimbs in females; the source of the discoloration was not certain although nasal bleeding was observed in a few instances. A short time after this phenomenon was noted, some of the animals failed to consume their daily food portion completely. This lack of appetite appeared first on diet 61 (5%), then on 62 (10%) and 60 (0%), and finally on 63 (20%) and 64 (40%). The animals fed the stock diet retained a vigorous appetite throughout the experiment. Finally during the last weeks of the experiments, animals began to die. This was not always simply the result of failure to eat, since a number of animals continued to eat regularly until they died. In other cases, however, animals failed to eat even after transfer to ad libitum feeding in large cages. These animals died during the thirteenth week (table 2).

There seems to be no obvious explanation for these observations. The lack of appetite suggests a deficiency of thiamine, but the diets used have supported excellent growth, reproduction and lactation when fed ad libitum. The superiority of the stock diet suggests the possibility that vitamins were present in the natural foods (wheat, oats, yeast, alfalfa) comprising this diet, or were synthesized by intestinal flora, which were not available to animals on the synthetic diets. It is possible that, under the special conditions of this experiment, significant alterations in intestinal synthesis might appear. We have often noted transient loss of hair on the head and shoulders, suggesting biotin deficiency, after transfer from restricted to ad libitum feeding. Experiments are now in progress to test these points further.

The results of the tests of physical capacity have been presented elsewhere (Scheer et al., '47a). They add no further illuminating details to the general picture; a marked decrease in endurance appeared in all groups but was not related to

fat content in the diet. Recovery was rapid and complete in all groups.

TABLE 2

The total mortality, bleeding and lack of appetite in young adult rats fed experimental diets at levels of 24 cal. per day for 8 weeks and 12 cal. per day for following 4 weeks.

WEEKS ON RESTRICTED DIET	RESULTS AFTER FOLLOWING DIETS									
	60B		61		62		63		64	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Total mortality										
10				4						
11	4		8	4	4	4			4	
12	35	21	67	32	56	29	24	16	4	4
13	65	58	75	44	70	50	52	36	12	20
Bleeding										
7						4				
8				12		13				
9				16		22				
10				12	4	22		4		
11			9	12	13	18	13	8		
Loss of appetite										
8			25	52						
9			29	52						
10	9	37	92	96	50	69	4			8
11	77	84	100	100	96	100	87	100	13	28
12	94	100	100	100	100	100	100	100	35	68

Recovery of body weight

At the end of 12 weeks, most of the surviving animals were returned to large cages and fed the same diets ad libitum. Several from each group, however, were sacrificed for determinations of body composition. The mean weights of the survivors with their standard errors, are presented in table 3. The high mortality, especially among the males, reduced the number of animals involved; consequently the differences are not always statistically significant; those for which the probability of a similar result by chance is less than 1 in 100 are tabulated at the right of the table. The superiority of

TABLE 3

Changes in weight of young adult rats surviving a 12-week period of severe caloric restriction during a subsequent period of ad libitum feeding.

DIET NO.	NO. OF RATS	BODY WEIGHT IN GM [1]					M.D. : S.E.M.D.[2]	
		Start	After 12 wks. restricted	Recovery period			3-wk. recovery	6-wk. recovery
				3 wks.	6 wks.	9 wks.		
Male rats								
60B [3]	4	273.0 ± 15.9	140.2 ± 8.5	173.2 ± 53.2	242.9 ± 12.9			63, 3.7; S., 2.5.
61	1	296	128	172	247	277		
62	1	250	130	248	283			
63	6	274.0 ± 12.2	159.7 ± 7.3	259.5 ± 8.3	304.7 ± 10.5	303.7 ± 12.9		
64	12	260.9 ± 10.2	140.4 ± 5.0	237.4 ± 8.0	291.6 ± 5.3			
S [4]	15	267.5 ± 5.6	147.7 ± 8.7	244.1 ± 6.0	286.9 ± 11.9			
Female rats								
60B [3]	7	187.9 ± 7.2	121.0 ± 4.0	171.4 ± 7.1			63, 4.8; 64, 3.3; S., 4.0.	
61	6	192.8 ± 7.5	112.8 ± 5.5	184.8 ± 4.4	212.7 ± 5.3	213.5 ± 7.9	63, 4.4; S., 3.1.	63, 2.7.
62	6	187.0 ± 8.2	122.2 ± 13.8	177.2 ± 10.0	203.2 ± 13.3	213.0 ± 16.6	63, 3.2; S., 2.5.	
63	10	191.1 ± 4.9	133.6 ± 4.9	211.5 ± 4.2	235.0 ± 6.2		61, 2.8.[5]	64, 2.6; S., 3.6.
64	13	192.1 ± 3.7	123.1 ± 3.7	198.5 ± 4.1	212.6 ± 5.8	213.6 ± 5.9		
S [4]	20	190.2 ± 3.5	134.3 ± 2.2	205.0 ± 4.7	205.4 ± 5.3	217.5 ± 6.2	60B, 4.5; 61, 3.6; 64, 2.6.[5]	

[1] Including the standard error of the mean calculated as follows $= \sqrt{\Sigma d^2/n}/\sqrt{n}$ where "d" is the deviation from the mean and "n" is the number of observations.

[2] Ratio of mean difference to standard error of mean difference. When this is 2.5, it indicates that the chances that such a difference might arise by chance are no more than 1 to 100.

[3] Containing 1.0% methyl linolate.

[4] Stock diet.

[5] Ratio M.D. : S.E.M.D. for 12-week restricted period.

diet 63 (20% fat) in supporting recovery of body weight is evident in the female series. It is of interest to note here that the stock diet, which supported rapid gain in weight to a value slightly above the weight at the beginning of the experiment, did not permit as great eventual gains as did diet 63.

Reproductive performance

Immediately after transfer to ad libitum feeding, 5 females from each dietary group were placed in separate large cages with males from the same group if these were available. Only 1 litter was born to animals subsisting on diets 60b and 62 and none on diet 61 while 5 litters were produced each by the females on diets 63 and 64 and 4 on the stock diet. None of the litters was successfully raised to weaning in spite of the fact that the number of young per litter was small. The results are in sharp contrast with those presented earlier (Scheer, '47b) in which most of the litters born to rats which had been subjected to caloric restriction immediately after weaning were reared successfully. Evidently recovery of reproductive capacity after such severe restriction is much less rapid than recovery of body weight or physical capacity.

DISCUSSION

The experiments presented in this series of papers (Deuel et al., '47; Scheer et al., '47a, '47b) have invariably resulted in the same conclusions; in every case, animals performed more satisfactorily when fed diets containing liberal amounts of fat than when fed diets containing little or no fat. It is possible to explain the growth effects with ad libitum feeding on the basis of increased caloric intake with higher fat content of the diet. The results with restricted isocaloric feeding cannot be so explained. The valuable observation of Forbes et al. ('46a, '46b) that the metabolic efficiency of food utilization is greater when fat is present suggests one important possible explanation. It does not seem however, that all of the effects which we have observed, i.e., on physical capacity, resistance to undernutrition, reproduction and lactation, can be the result

simply of increased efficiency of food utilization. Rather, it appears that fat must take an active part in a variety of fundamental vital processes underlying the phenomena which we have observed. Increased fat in the diet, then, would be expected to increase the effectiveness of all of these processes by providing a readily available stock of fat, thus obviating the relatively expensive necessity of fat formation from carbohydrate. In this view, fat is no longer the inert storage material of the older biochemistry, but an active component of the metabolic machinery.

SUMMARY

When young adult rats are subjected to severe caloric restriction on diets varying in fat content, weight loss is less rapid, and mortality is less, on diets containing liberal amounts of fat than on a fat-free diet. During recovery from the period of caloric restriction, weight loss is more rapidly regained, and reproductive capacity is superior on diets containing fat. Reproductive capacity is still subnormal 4 to 6 weeks after transfer to ad libitum feeding, a time when body weight and physical capacity have returned to normal. The significance of the results of the present series of experiments is briefly discussed in relation to the role of fat in metabolism.

LITERATURE CITED

DEUEL, H. J., JR., E. R. MESERVE, E. STRAUB, C. HENDRICK AND B. T. SCHEER 1947 The effect of fat level of the diet on general nutrition. I. Growth, reproduction and physical capacity of rats receiving diets containing various levels of cottonseed oil or margarine fat ad libitum. J. Nutrition, *33:* 569.

FORBES, E. B., R. W. SWIFT, R. F. ELLIOTT AND W. H. JAMES 1946a Relation of fat to economy of food utilization. I. By the growing albino rat. J. Nutrition, *31:* 203.

———— 1946b Relation of fat to economy of food utilization. II. By the mature albino rat. J. Nutrition, *31:* 213.

SCHEER, B. T., S. DORST, J. F. CODIE AND D. F. SOULE 1947a Physical capacity of rats in relation to the energy and fat content of the diet. Am. J. Physiol. (in press).

SCHEER, B. T., D. F. SOULE, M. FIELDS AND H. J. DEUEL, JR. 1947b The effect of fat level of the diet on general nutrition. II. Growth, mortality and recovery in weanling rats maintained on restricted calories. J. Nutrition, *33:* 583.

NUTRITIONAL SURVEYS IN WESTERN HOLLAND: ROTTERDAM, 1945 [1]

CYRUS E. FRENCH [2] AND FREDRICK J. STARE [3]

Institute of Animal Nutrition, The Pennsylvania State College, State College, Pa.; and Department of Nutrition, Schools of Public Health and Medicine, Harvard University, Boston, Massachusetts

ONE FIGURE

(Received for publication February 8, 1947)

INTRODUCTION

During the course of the war the Nutrition Division, Office of the Surgeon General, U.S. Army, under the direction of Col. John B. Youmans, formulated detailed plans for nutritional surveys of civilian populations in liberated and occupied countries. When the defeat of the German armies in the Netherlands appeared imminent early in March, 1945, a survey team was requested by the Public Health Branch of Supreme Headquarters Allied Expeditionary Forces and was immediately dispatched to that area. This team joined a similar Canadian group headed by Wing Commander J. M. McCreary in performing a series of 1-day surveys of newly liberated cities and towns during the advance into southern Holland with the First Canadian Army. These rapid surveys were designed to furnish Civil Affairs detachments with immediate information about local food supplies and nutritional conditions.

[1] Presented at the meetings of the Agricultural and Food Division, American Chemical Society at Chicago, September 9–12, 1946.

[2] On leave; Captain, Sn.C., Army of the United States.

[3] Consultant to the Surgeon General, United States Army.

Each survey team consisted essentially of the following: a medical officer with special training in deficiency diseases; a nutrition officer with special training in obtaining dietary histories, and in conducting biochemical studies; a senior Dutch medical student who acted as interpreter and assistant; and clerical personnel. The American team was equipped with a portable laboratory designed and constructed by the senior author with the cooperation of Drs. R. E. Johnson ('45), O. A. Bessey and O. H. Lowry ('45), for making certain laboratory studies in the field such as hemoglobin, serum protein, plasma ascorbic acid, and urinary excretion tests for thiamine, riboflavin, nicotinic acid, chlorides, albumin and ketone bodies. It proved impractical however to attempt laboratory investigations in connection with the rapid surveys because of the emphasis on speed and movement. When detailed surveys were carried out in the cities of western Holland much biochemical work was done.

The First Canadian Army was moving northward into central Netherlands against very determined resistance during April and early May, 1945. The British Second Army meanwhile had swung to the east and moved rapidly northward along the Dutch German border, thus effectively cutting off a large number of German forces from their bases of supply. As the German forces and large numbers of inhabitants were steadily forced back into the smaller, more densely populated, and less agriculturally productive area of western Holland reports of hunger and starvation in the civilian population were received in ever increasing numbers. Although unconfirmed, such reports seemed logical in view of the general overcrowded condition and the customary practice of German troops to live off the land. Accordingly, detailed plans and procedures of operation for the assessment of nutritional conditions and possible relief measures were formulated with Dutch, British and American authorities by the nutrition branch of SHAEF Mission Netherlands under the direction of Lt. Col. H. R. Sandstead, U.S. Public Health Service.

Following the capitulation of the German military forces in western Holland on May 7, 1945, similar rapid surveys were made in that area to determine the immediate needs of the population for emergency food supplies, medical care and for aid from Netherlands Red Cross feeding teams. In general only poor sections of the cities were sampled in these preliminary studies and nutritional conditions were found to be much more severe than in southern and eastern Holland. By the time these studies were completed on May 18, 1945, conditions had improved considerably. Emergency food supplies were being distributed and Netherlands Red Cross feeding teams were setting up and operating a number of emergency hunger wards in many of the hospitals.

The Canadian and American survey teams were then joined by a British unit and mobile laboratory headed by Dr. H. M. Sinclair and each of the teams was augmented with Dutch physicians, biochemists, dietists, secretarial and other personnel. Detailed surveys were then undertaken in Leiden and The Hague by the British team, in Utrecht and Amsterdam by the Canadian team and in Delft and Rotterdam by the American team. Nearly 14,000 persons of all ages and economic groups were surveyed in these comprehensive studies which were completed by the end of June, and definite evidence of widespread starvation was recorded (Burger et al., '45). Results were used to advise allied authorities regarding the kinds and quantities of food needed during the period of recuperation; to advise and support Dutch food and health authorities; and to determine the effects of extended underfeeding on the entire population. With reference to the latter purpose it should be mentioned that a monograph is being prepared by Dutch, British, Canadian and American investigators which will constitute a comprehensive scientific report of the causes, the course of events, and the results of mass starvation in Holland.

The data to be presented and discussed in this report will be limited to those obtained in surveys of Rotterdam on May 9 and again during the period May 29 to June 8, 1945. They may

be considered to be representative generally of conditions that existed at this time in the other great cities of western Holland as judged by the findings of the other teams. Rotterdam is the principal seaport and third largest city of the Netherlands. The normal population of 600,000 remained unchanged despite the severe German bombings in 1940 that destroyed large areas in the heart of the city and that resulted in considerable overcrowding and unemployment. It was selected for a detailed investigation following the rapid survey of May 9, 1945, which had indicated a very poor nutritional condition of the population.

PROCEDURE

A representative sample of the population which included all age groups, both sexes, various occupations and economic levels was selected in the following manner. An organization meeting was held in the office of the Director of the City Health Service on May 29th to explain the general plan and purpose of the survey. Representatives of the ration distribution office, the volunteer women's organization, the domestic science schools and the public health service attended this meeting, and definite plans were developed for conducting the survey. It was agreed that a total of approximately 2500 people should be examined and that these should be divided on the basis of 50% from the poor, 40% from the middle, and 10% from the upper classes to be representative of the entire population. Experience with previous surveys had shown that approximately 250 persons could be examined in 1 day at 1 location, hence 5 poor areas of the city, 4 middle class areas, and 1 upper class area were chosen carefully to assure scattering of the sample throughout the city area. Each such area contained approximately 2000 people so that a valid sample was obtained.

A second conference was held on the following day in the ration distribution office to consolidate plans for obtaining complete information on the past and present food ration and all other available food. Information obtained during

previous surveys indicated that the food situation had been steadily deteriorating during the German occupation until the autumn of 1944 when it became acute due to a national railway strike and the resulting punishment policy that was inflicted by the oppressors (Stare, '45). It was hoped that quantitative estimates of actual food consumption during 3 progressive periods, October, 1944, February, 1945, and April, 1945, might be obtained in order to establish a valid sequence of data leading up to the present survey. This plan was accomplished through the efforts of the household science teachers of the city who questioned approximately 90 families, in proper proportion, from poor, middle and upper class communities with regard to all food consumed during those months. Arrangements were also made to obtain records of food distributed by the Central Kitchen Organization, the Inter-Church Organization, and the Red Cross.

Personnel in a representative sample of the hospitals, rest homes, old folks homes, orphanages, prisons, and asylums, as well as in all 9 emergency hunger hospitals were also examined.

The actual examination of the general population was conducted at a prearranged time and place in each of the 10 sections of the city. Here the volunteer women's organizations were found to be invaluable not only in contacting the chosen families and seeing that they reported for examination at the proper time, but also in assisting with the recording of data and in maintaining the even flow of patients through the examining rooms. At the start of the examination each person was presented with a record card that contained the necessary blank spaces for entry of personal data, subjective complaints, height, weight, age, physical examination data, and laboratory determinations to be recorded as the bearer progressed through the clinic. In about 10% of the cases selected at random, complete dietary histories of food consumed during the past 24 hours and finger blood samples for hemoglobin and serum protein determinations by the gravity gradient method were obtained. In addition, approximately 100 venous blood

samples were drawn at random from adult personnel and were analyzed by the mobile laboratory for plasma vitamin A, plasma carotene, ascorbic acid, hematocrit, white cell volume, hemoglobin, and total serum protein.

RESULTS

In the rapid and detailed surveys of Rotterdam which were conducted on May 9, 1945, and May 29 to June 8, respectively, nutritional conditions were found to be very severe with large numbers of the population exhibiting evidence of insufficient food consumption. Eighty-six per cent of 154 subjects examined in the first survey were classified as thin, very thin, or emaciated with an estimated adult weight loss of 30 pounds. Hunger edema was found frequently, being recorded in nearly 20% of those examined. Children between the ages of 1 to 12 years and adults over 60 years of age were in the poorest nutritional condition, with the aged most severely affected. It was estimated from the survey data as well as by public health and other medical personnel that there were in the city 40,000 cases showing some degree of famine edema. Many deaths due to starvation were stated to have occurred, the total number being unavailable at the time of the studies. However, the death rate during the period January to April, 1945, had increased more than 100% over that of the previous year (table 1). Evidences of specific vitamin deficiencies were rarely noted. The average daily food consumption at the time of the early survey was approximately 1000 Cal., with a range of 500–1500 Cal. The dietary consisted mainly of coarse bread, potatoes, sugar beets, other available fresh vegetables, and a very limited amount of cereals, dairy products and meat.

In the detailed survey conducted from May 29 to June 8, 1945, a total of 2660 people, selected to be representative of the general population, were examined. In addition, 185 persons were examined in 20 institutions chosen to be representative of the hospitals, asylums, orphanages and old people's homes. Most of the hospitalized hunger patients were con-

centrated in emergency hunger hospitals, and hardly without exception these patients showed severe cachexia. Patients in other hospitals did not present a major problem in underfeeding.

TABLE 1

General mortality — Rotterdam (January–April).

	1944	1945	INCREASE (%)
Infants (0–1 yr.)	126	319	153
Children (1–5 yrs.)	78	173	122
Adults (5–65 yrs.)	910	2314	154
Males	495	1715	246
Females	415	599	44
Adults (65 + yrs.)	1179	3148	167
Males	537	2019	276
Females	642	1129	76
Total	2293	5952	159
Males	1159	4032	248
Females	1134	1920	69
	1944	1945	INCREASE (%)
Death rate (per 1000) — Rotterdam	11.5	29.8	159
Death rate (per 1000) — Boston	12.9	13.0	1

The grading in physical appearance of the people examined on May 9th as compared with those examined in the later survey is shown in table 2. An improvement in physical appearance, apparently took place in the interval between the 2 surveys. The 2 groups, however, were not strictly comparable in that the early survey was conducted in a relatively poor area of the city while the latter more detailed study included representative groups of all economic means. In grading the physical appearance scores in the latter survey approximately 40% of the poor people were judged normal, 50% of the middle class and 80% of the upper class. The estimated weight loss averaged 25 pounds in the groups from 19 to 59 years of age and 40 pounds in those over 60 years, with females showing slightly more loss than males.

The symptoms of weakness, fatigue, diarrhea, muscle pain and paresthesia were prevalent in all groups, especially in the aged. Amenorrhea was frequently reported.

Papillary atrophy, pallor, and follicular hyperkeratosis were the commonest clinical findings of nutritional interest occurring in 20 to 80% of the people and to a greater degree in those from the poor districts. Edema was present in about 3% of the people from the poor districts in the 19– to 59-year age group, and in nearly 12% of those over 60 years of age. This shows a considerable decrease in the incidence of

TABLE 2

General physical appearance — Rotterdam (May 9, 1945; May 29–June 8, 1945).

	TOTAL NO. EXAMINED	NORMAL	FAIR	POOR	SEVERE
		%	%	%	%
Adults	May 154	14	36	44	6
	June 1511	48	46	6	0
Children	May 118	10	31	31	28
	June 1149	22	44	34	0
"Hunger patients"					
Adults	May 127	0	0	25	75
	June 242	0	6	76	18
Children	May none	..	..	..	..
	June 380	0	5	62	33

edema as compared to the 20% average observed in the earlier survey.

Hemoglobin and total serum protein values, summarized in table 3, varied from 11 to 14 gm per 100 ml and 6 to 7.6 gm per 100 ml, respectively, with little difference between economic groups. Plasma volume was determined in the emergency hunger hospitals and was found to be 20 to 30% below normal in hunger patients. Average hemoglobin and total serum protein values in this group were only slightly below the general population averages although much greater variation was noted.

In addition to the finger blood determinations, approximately 100 venous samples were obtained at random from adult males and females and were analyzed in the mobile laboratory with the following results: hematocrit 41.4 volume %; white cell volume 1.0 volume %; hemoglobin 13.4 gm per 100 ml; serum protein 7.5 gm per 100 ml; plasma vitamin A 108 I.U. per 100 ml; plasma carotene 181 I.U. per 100 ml; and whole blood ascorbic acid 0.37 mg per 100 ml. Values obtained from different economic groups showed no significant variation.

TABLE 3

Average hemoglobin and serum protein values — Rotterdam (May 29–June 8, 1945)

AGE	NUMBER OF DETERMINATIONS	HEMOGLOBIN	SERUM PROTEIN
years		*gm/100 ml*	*gm/100 ml*
	Males		
1–12	25	12.5	7.1
13–18	17	13.1	7.3
19–59	57	13.8	7.4
60 +	17	12.5	6.9
	Females		
1–12	24	12.2	7.0
13–18	11	12.5	7.5
19–59	77	12.2	7.4
60 +	8	12.0	7.5

The average daily consumption of nutrients by the 3 economic groups is presented in table 4 and represents nearly 100% of the official food ration at that time. It should be noted that the food intake increased from 1000 to 2000 Cal. in the 4-week period between the 2 surveys. Simultaneously the official ration rose from 340 to 2000 Cal. This was due largely to the rapid import and distribution of Allied food supplies. Food was distributed mainly through the Rotterdam Food Distribution Office with some additional quantities handled through the Central Kitchen, Inter-Church and Red Cross organizations.

Obviously the daily food consumption of approximately 2000 Cal. at the time of the detailed survey did not account for the clinical evidences of undernutrition found. Likewise, it would be inaccurate to ascribe the nutritional condition of the population to the daily consumption of 1000 Cal. at the time of the rapid survey 1 month earlier. In an effort to obtain at least a rough estimate of the successive dietary levels which were responsible for the conditions found in the 2 surveys, dietary histories of food consumption during 3 separate

TABLE 4

Average daily nutrient consumption — Rotterdam (May 9, 1945; May 29–June 8, 1945).

NUMBER QUESTIONED	CALORIES	PROTEIN	FAT	CHO	Ca	Fe	VIT. A	B_1	B_2	NIACIN	C
		gm	*gm*	*gm*	*gm*	*mg*	*I.U.*	*mg*	*mg*	*mg*	*mg*
1 poor class district: May 9, 1945											
27	1044	46	5	204	0.5	8	2600	0.9	0.9	13	164
5 poor class districts: June 1–8, 1945											
152	1851	53	45	243	0.7	12	6440	1.2	1.3	9	85
4 middle class districts: June 1–8, 1945											
77	1965	58	48	268	0.6	15	9840	1.4	1.4	12	100
1 upper class district: June 1–8, 1945											
21	2360	74	77	345	1.0	17	12900	2.0	1.6	13	169

monthly periods were obtained by the household science teachers. October, 1944, February and April, 1945, were chosen upon the advice of city authorities to represent successively the period preceding extreme food shortage, the winter period of hunger, and the period of extreme starvation prior to liberation. Average food consumption, as shown in figure 1, decreased from about 1700 Cal. to 1400 Cal. from the fall of 1944 to the spring of 1945 and then fell abruptly to about 1000 Cal. at the time of liberation as estimated by the rapid survey on May 9, 1945.

Although all food consumption data obtained through dietary questioning are subject to considerable error it should be

noted that the values obtained parallel the actual published official rations for the same periods. In a test of accuracy held in Paris a few months previous to this work, the dietary questioning technique used in the surveys was found to yield results within 10% of actual consumption figures.

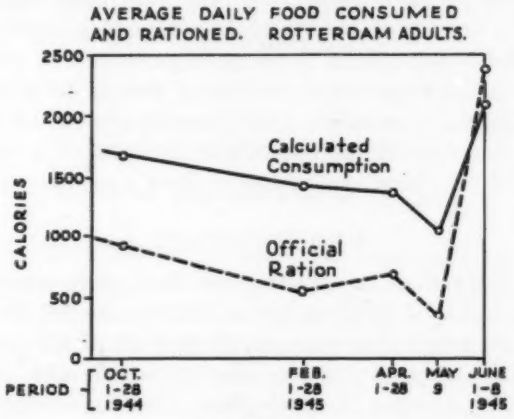


Figure 1

SUMMARY

Clinical, biochemical, and dietary data obtained in 2 nutritional surveys of Rotterdam, Holland, on May 9, 1945, and May 29 to June 8, 1945, are presented. A total of nearly 3000 people were examined.

In physical appearance 86% were classified as thin, very thin or emaciated in the early survey, and approximately 50% in the detailed survey 1 month later. Weight loss averaged about 25 pounds in the 19– to 59–year age group and 40 pounds in those over 60 years of age.

Symptoms of weakness, fatigue, diarrhea, muscle pain and paresthesia were prevalent, especially in the aged.

Papillary atrophy, pallor and follicular hyperkeratosis were common clinical findings.

Hunger edema was present in 20% of the people from poor class districts in the early survey, and in 3% and 12% of the

19- to 59-year age group and those over 60 years of age, respectively, in the later study.

Hemoglobin and total serum protein values averaged 12 to 14 gm per 100 ml and 7.0 to 7.5 gm per 100 ml, respectively.

Estimates of daily food consumption in 3 successive periods prior to the surveys were 1700, 1400, and 1400 Cal., respectively. The survey of May 9, 1945, indicated a further reduction in food consumption to an average intake of about 1000 Cal. The dietary consisted mainly of bread, potatoes, sugar beets and other vegetables. Considerable quantities of Allied food supplies were being distributed at the time of the second survey and the food consumption then averaged 2000 Cal. per day.

ACKNOWLEDGMENT

The authors wish to acknowledge their deep appreciation, especially to Lieut. Willem Nolen, Dr. von Andel, Dr. C. Den Hartog, and associates who contributed all of their time and energy to the successful prosecution of the surveys, and to the participating groups in Rotterdam for their wholehearted cooperation.

The leadership of Major Marvin B. Corlette, M.C., during the period of rapid surveys prior to May 7, 1945, is gratefully acknowledged.

LITERATURE CITED

BURGER, G. C. E., H. R. SANDSTEAD AND SIR JACK DRUMMOND 1945 Starvation in Western Holland. Lancet, Sept. 1, p. 282.

JOHNSON, R. E. 1945 Laboratory Manual of Field Methods. Harvard Fatigue Laboratory, Boston, Massachusetts.

LOWRY, O. H., AND T. H. HUNTER 1945 The determination of serum protein concentration with a gravity gradient tube. J. Biol. Chem., *159:* 465.

STARE, F. J. 1945 Nutritional conditions in Holland. Nutrition Revs., *3:* 225.

THE INFLUENCE OF AUTOCLAVING SOYBEAN OIL MEAL ON THE DIGESTIBILITY OF THE PROTEINS[1]

ROBERT JOHN EVANS, JAMES McGINNIS AND J. L. ST. JOHN
Divisions of Chemistry and Poultry Husbandry, Washington Agricultural Experiment Stations, Pullman

(Received for publication November 12, 1946)

That heating influences the nutritive value of soybean proteins has been recognized for several years. Evans and McGinnis ('46) reviewed the literature on this problem. They also presented additional data showing that moderate heating of raw soybean oil meal[2] increased its nutritive value for the growing chick. However, when such a meal was autoclaved at 130°C. for 30 or 60 minutes, the nutritive value was decreased. Ham and Sandstedt ('44) and Bowman ('44) have reported the presence of a trypsin inhibitor in soybean oil meal which is destroyed by heat. They believe that destruction of this enzyme inhibitor by heating accounts for the increased nutritive value of heated soybean oil meal. Evans ('46) studied the influence of autoclaving soybean oil meal on the liberation of amino groups from the soybean oil meal by different enzymes and enzyme combinations and found that when trypsin or trypsin and erepsin were used, a marked increase in digestibility resulted from autoclaving the soybean oil meal at 100°C. for 30 minutes. When treatment with

[1] Published as Scientific Paper no. 693, College of Agriculture and Agricultural Experiment Stations, Institute of Agricultural Sciences, State College of Washington, Pullman, Washington.

[2] The raw soybean oil meal used by Evans and McGinnis ('46) and that used in the present study was prepared by grinding solvent-extracted soybean flakes in a hammer mill followed by a thorough mixing.

pepsin preceded these digestions, no differences were observed. In all cases, autoclaving at 130°C. for 60 minutes very markedly decreased the liberation of amino groups by any of the enzyme combinations.

It was felt desirable to study further the digestibility of soybean oil meal proteins as influenced by autoclaving. The criteria chosen were digestibility of the proteins and sulfur compounds. The sulfur digestibility was considered to be especially important since Almquist, Mecchi, Kratzer and Grau ('42) believed methionine to be the principal growth limiting deficiency in soybean proteins. It is the purpose of the present paper to report the influence of the autoclaving of soybean oil meal upon the digestibility of total protein, the sulfur-containing proteins, the cystine-containing proteins, and the methionine-containing proteins as determined by the quantity of protein, sulfur, cystine and methionine remaining in the undigested protein fraction. Three digestion procedures were used: (1) the chick, (2) *in vitro* pepsin, trypsin, and erepsin, and (3) *in vitro* trypsin, and erepsin. Using chick data as a standard, comparisons were made with the 2 *in vitro* digestion procedures in an attempt to determine which most nearly approached the chick digestion. Two comparisons were made: (1) of the actual percentage of undigested protein and (2) of the effect of autoclaving on the raw protein. A graph in which the percentage of undigested protein is plotted against the autoclaving temperature best shows this second comparison. If the graph of one of the *in vitro* enzyme digestions parallels that for the chick digestion the 2 may be said to be affected similarly by the autoclaving treatment of the soybean oil meal. Such a parallel between curves is most readily shown by means of a coefficient of correlation calculated from the data presented in table 1 between the protein of the 6 soybean oil meals not digested by the chicks and that not digested by either of the *in vitro* enzyme combinations. For each coefficient of correlation 6 values (1 from each soybean oil meal) for the enzyme digests were correlated with 6 group values for chick digestion.

EXPERIMENTAL

This experiment is a continuation of the work reported earlier by Evans and McGinnis ('46). The data reported in the present paper were obtained from the 6 groups of 15 chicks each that received, in addition to the basal diet, 0.2% choline and were designated as groups 4 to 9 in the earlier study. Evans and McGinnis ('46) have described the methods of preparing the soybean oil meals, the diets fed, care of chicks, and collection of the droppings for chemical analyses. Each group of chicks was considered as a unit in the balance studies. All nitrogen determinations were made by the Kjeldahl-Gunning-Arnold method (A.O.A.C., '45). Uric acid nitrogen was determined by the method of St. John and Johnson ('31). Total sulfur was determined as described by Evans and St. John ('44), and organic sulfur as described by Evans and Greaves ('37). Cystine and methionine were determined by the differential oxidation procedure (Evans, '45b).

The nitrogen precipitated from the droppings by trichloroacetic acid minus the uric acid nitrogen in the droppings was considered to be the nitrogen not digested by the chick. It was necessary to subtract the uric acid nitrogen from the trichloroacetic acid precipitated nitrogen because uric acid is insoluble in acid solutions. Undigested organic sulfur, cystine and methionine were considered to be the amounts of these materials in the droppings precipitated by or insoluble in a 5% solution of trichloroacetic acid. To determine the trichloroacetic acid precipitated fraction 2.0 gm of the droppings were suspended by stirring with 50 ml of 5% trichloroacetic acid. The insoluble material was filtered on a no. 42 Whatman Filter Paper, washed 3 times with hot water containing a little trichloroacetic acid and the desired determination made on the residue. From the nitrogen intake of the group from the total diet, during the 1 week duration of the experiment, and the amount of undigested nitrogen in the droppings from the group, the per cent of nitrogen in the diet that was not digested was calculated. Similar calculations

were made to determine the per cent of the organic sulfur, cystine and methionine in the undigested protein fraction.

In vitro enzyme digestions were carried out as described by Evans ('46) using pepsin, trypsin and erepsin as 1 enzyme combination, and trypsin and erepsin as the other. Trichloroacetic acid precipitations were made of these digests as described by Evans ('46). The per cent glutelin in the different heat-treated soybean oil meals was determined by the method of Lund and Sandstrom ('43).

RESULTS AND DISCUSSION

The influence of the autoclaving of soybean oil meal on the digestibility of protein is shown in table 1. In calculating the percentage of protein in the soybean oil meal not digested by the chick, the assumption was made that all of the undigested protein was from the soybean oil meal and that protein from all other sources was completely digested. The validity of this assumption may be questioned, but it is reasonable to assume that the same percentage of protein from the other sources (5% brewer's yeast and 5% gelatin) was digested by all groups. The same method of calculation was used for organic sulfur, cystine sulfur and methionine sulfur.

Autoclaving raw soybean oil meal at 100–130°C. for 30 minutes increased digestion of the proteins by the chick and by trypsin and erepsin *in vitro*, but did not increase protein digestion by pepsin, trypsin and erepsin *in vitro*. Autoclaving at 130°C. for 60 minutes, in all cases, decreased protein digestion from that obtained after less severe autoclaving. A comparison of the per cent total protein not digested by the chick and that not digested by *in vitro* enzyme combinations shows that the chick digestion in magnitude more nearly approached that of pepsin, trypsin, and erepsin *in vitro* than that of trypsin, and erepsin. The chick digested the protein less completely than pepsin, trypsin, and erepsin *in vitro* and more completely than trypsin and erepsin *in vitro*. A highly significant coefficient of correlation of $+0.998$ between the soybean oil meal protein not digested by the chick and that

TABLE 1

The influence of autoclaving soybean meal on the digestibility of the proteins.

Heat treatment of soybean oil meal						
Time, *minutes*	none	30	30	30	30	60
Temperature, °*C.*	none	100	110	120	130	130
Protein digestibility data with the chick [1]						
Nitrogen intake from soybean oil meal, *gm*	22.6	45.2	38.8	43.1	31.1	21.8
Nitrogen ppt. from droppings by trichloroacetic acid, *gm*	16.9	30.0	25.7	25.4	20.5	14.8
Uric acid nitrogen, *gm*	11.7	24.8	21.7	21.2	16.9	11.3
Undigested nitrogen,[2] *gm*	5.2	5.2	4.0	4.2	3.6	3.5
Undigested protein in soybean oil meal,[3] %	23.0	11.5	10.3	9.7	11.6	16.1
Soybean oil meal protein not digested by in vitro enzyme digestion						
Pepsin, trypsin, erepsin, %	10.9	6.7	7.8	10.2	11.0	20.7
Trypsin, erepsin, %	34.9	15.9	12.5	12.4	15.4	23.1
Organic sulfur digestibility data with the chick						
Organic sulfur intake from soybean oil meal, *gm*	1.16	2.31	1.98	2.20	1.59	1.11
Organic sulfur ppt. from droppings by trichloroacetic acid, *gm*	0.57	0.74	0.68	0.65	0.54	0.49
Undigested organic sulfur in soybean oil meal,[3] %	49.1	32.0	34.3	29.5	34.0	44.1
Organic sulfur in soybean oil meal protein not digested by in vitro enzyme digestion						
Pepsin, trypsin, erepsin, %	19.4	24.4	23.6	31.3	29.2	40.8
Trypsin, erepsin, %	45.6	28.1	26.0	27.5	29.4	38.2
Cystine digestibility data with the chick						
Cystine sulfur intake from soybean oil meal, *gm*	0.71	1.42	1.22	1.35	0.98	0.68
Cystine sulfur ppt. from droppings by trichloroacetic acid, *gm*	0.46	0.64	0.56	0.58	0.49	0.39
Undigested cystine sulfur in soybean oil meal,[3] %	64.8	45.1	45.9	43.0	50.0	57.4
Cystine in the soybean oil meal protein not digested by in vitro enzyme digestion						
Pepsin, trypsin, erepsin, %	21.2	29.9	28.1	35.1	40.7	49.8
Trypsin, erepsin, %	50.6	35.9	33.8	30.3	32.0	41.6
Methionine digestibility data with the chick						
Methionine sulfur intake from soybean oil meal, *gm*	0.45	0.89	0.76	0.85	0.61	0.43
Methionine sulfur ppt. from droppings by trichloroacetic acid, *gm*	0.10	0.06	0.08	0.05	0.04	0.11
Undigested methionine sulfur in soybean oil meal,[3] %	22.2	6.7	10.5	5.9	6.6	25.6
Methionine in the soybean oil meal protein not digested by in vitro enzyme digestion						
Pepsin, trypsin, erepsin, %	16.4	15.8	16.4	25.3	16.4	26.7
Trypsin, erepsin, %	37.7	15.8	13.7	23.3	25.3	32.9

[1] Each group contained 15 chicks.

[2] Undigested nitrogen was the nitrogen precipitated by trichloroacetic acid minus the uric acid nitrogen.

[3] These values are calculated by making the assumption that all of the protein (nitrogen) other than that from the soybean oil meal was completely digested and hence that all of the undigested protein was from the soybean oil meal.

not digested by trypsin and erepsin *in vitro* and a non-significant correlation + 0.364 between the protein not digested by the chick and that not digested by pepsin, trypsin, and erepsin *in vitro* were obtained. These data indicate that, although in most cases the percentages of protein not digested by the chick were closer to the pepsin, trypsin, and erepsin values, because of the raw meal if the values are plotted against heat treatment the chick curve more nearly approaches the trypsin and erepsin curve. These data are interesting in view of the statement by Schoening ('39): "Strictly speaking, there is no true gastric digestion in the fowl such as is common to mammals." A poor pepsin activity in the chicken might well be expected. It appears probable, however, that some pepsin activity or other activity may be present in the chick because of greater digestion by the chick than by the enzymes trypsin and erepsin alone.

Data are presented in table 1 on the influence of autoclaving soybean oil meal on the per cent of the organic sulfur remaining in the undigested protein fraction. Autoclaving the raw meal for 30 minutes at 100–130°C. increased digestibility of the sulfur containing proteins for the chick and for trypsin and erepsin *in vitro,* but decreased it for pepsin, trypsin, and erepsin *in vitro.* Autoclaving for 60 minutes at 130°C. in all cases gave a soybean oil meal in which more of the sulfur was present in the undigested protein than when the meal was autoclaved less drastically. *In vitro* enzyme digestion with trypsin and erepsin or with pepsin, trypsin, and erepsin (1 exception) gave more complete digestion of the sulfur compounds than did digestion by the chick. In magnitude the *in vitro* trypsin and erepsin digestion more nearly corresponded to the chick digestion. A coefficient of correlation between organic sulfur not digested by the chick and that not digested by *in vitro* trypsin and erepsin digestion of + 0.959 was obtained. A value of 0.917 was necessary for the results to be highly significant. The correlation coefficient between *in vitro* pepsin, trypsin, and erepsin digestion and digestion by the chick was + 0.031. Autoclaving soybean oil meal for

30 minutes at 100°C. decreased the amount of organic sulfur in soybean oil meal not digested by the chick from 49% to 32%. This is of interest since Johnson, Parsons and Steenbock ('39) observed no differences in digestibility of sulfur compounds in raw and cooked soybean oil meals, as determined by sulfur balance determinations with adult rats. Evans and McGinnis ('46) observed an increase in retention of organic sulfur as a result of autoclaving raw soybean oil meal. It appears, therefore, that the adult rat may be able to digest the sulfur-containing proteins of raw soybean oil meal to a greater extent than can the growing chick. This might be due to differences between the type of protein digestion that prevails in the growing chick as compared to the adult rat. Pepsin in the adult rat might digest sulfur-containing proteins that would otherwise not be acted upon. This could well be explained on the basis of a digestion by pepsin of the trypsin inhibitor present in soybean oil meal which was shown by Kunitz ('45) to be a protein. Ham, Sandstedt and Mussehl ('45) demonstrated that the trypsin inhibitor when added to chick diets retarded growth of the chicks. McGinnis and Menzies ('46) showed that *in vitro* papain digestion improved the nutritive value of raw soybean flakes as effectively as did autoclaving for chicks. Carver, McGinnis, McClary and Evans ('46) observed equally good egg production of hens receiving raw and those receiving heat-treated soybean oil meal as the protein concentrate.

Data concerning the influence of autoclaving soybean oil meal on the per cent of the total cystine and methionine in the undigested protein fraction are presented in table 1. Autoclaving the raw soybean oil meal at 100–130°C. for 30 minutes increased digestibility of the cystine and methionine-containing proteins for the chick and for trypsin and erepsin *in vitro*. Digestibility of the cystine-containing proteins for pepsin, trypsin and erepsin *in vitro* was decreased by autoclaving, but the digestibility of the methionine-containing proteins by this enzyme combination was not affected except at higher autoclaving temperatures which in 2 cases decreased digestibility.

A highly significant coefficient of correlation of + 0.936 between cystine sulfur of soybean oil meal not digested by the chick and that not digested by trypsin and erepsin *in vitro* was obtained. Low correlation (+ 0.108) was observed between the chick digestion of cystine-containing proteins and pepsin, trypsin and erepsin digestion *in vitro*. As with organic sulfur, the *in vitro* digestion of cystine-containing proteins by trypsin and erepsin or pepsin, trypsin, and erepsin was more complete than was digestion by the chick. In magnitude the *in vitro* trypsin and erepsin digestion most nearly approached the chick digestion. Autoclaving the raw soybean oil meal at 130°C. for 60 minutes more than doubled the cystine in this protein fraction and nearly doubled the protein. Also of interest was the observation that the cystine-containing proteins of the soybean oil meals which were autoclaved at 120°C. or higher were more completely digested *in vitro* by trypsin and erepsin than by pepsin, trypsin and erepsin, though trypsin and erepsin digested the uncooked cystine-containing proteins or those cooked at lower temperatures to a lesser extent than the pepsin, trypsin, and erepsin combination. No explanation for these results can be offered at this time although it might be in some way tied up with heat denaturation and its effect on the —SH and —S—S— groups. It is recognized that heat denaturation in some way changes the activity of these groups (Anson, '45).

The chick digested a larger proportion of the ingested methionine present in any of the soybean oil meals than was digested *in vitro* by trypsin and erepsin, and digested more from all of the meals except the raw one than was digested *in vitro* by pepsin, trypsin and erepsin (table 1). These values for the chick are of especial interest because of the low percentage of methionine in the undigested protein fraction except for the raw soybean oil meal and the one that was autoclaved for 60 minutes at 130°C. The *in vitro* pepsin, trypsin, and erepsin values more nearly agree with the chick values in magnitude. A coefficient of correlation of + 0.766 was obtained between the per cent of soybean oil meal methi-

onine not digested by the chick and that not digested by trypsin and erepsin. One of 0.811 was required for significance. Neither the cystine nor methionine undigested by pepsin, trypsin, and erepsin were correlated with those undigested by the chick. It appears evident from the data presented in table 1 that soybean oil meal contains a protein high in cystine content which is poorly digested by the chick or by the *in vitro* enzyme combinations used. On the other hand, the percentage of the total methionine not digested by the chick or by *in vitro* enzyme digestion is in the same magnitude as the percentage of the total protein not digested in these ways

TABLE 2

The influence of autoclaving soybean oil meal on the nutritive value for chicks, per cent glutelins, and amino groups liberated by in vitro enzyme digestion.

HEAT TREATMENT OF SOYBEAN OIL MEAL		GAIN IN WEIGHT OF CHICKS TO 4 WEEKS [1]	PROTEIN EFFICIENCY [1]	GLUTELINS	AMINO GROUPS LIBERATED BY IN VITRO ENZYME DIGESTION	
Time	Temp.				Pepsin, trypsin, erepsin [2]	Trypsin, erepsin [2]
min.	*°C.*	*gm*	*gain/protein*	*%*	*%*	*%*
None		76	1.24	25.4	35	22
30	100	174	1.81	49.0	36	31
30	110	156	1.76	56.7	36	32
30	120	162	1.66	36.5	37	33
30	130	120	1.57	18.8	33	28
60	130	69	1.09	7.0	29	24

[1] These values are taken from the paper by Evans and McGinnis ('46).
[2] These values are taken from the paper by Evans ('46).

(table 1). Melnick, Oser and Weiss ('46) suggest that the difference in nutritive value of raw and autoclaved soybean oil meals is due to a difference in rate of liberation of methionine. Data in table 1 indicate that the amount of methionine remaining in the protein fraction not digested by the chick is greater for raw than for properly autoclaved soybean oil meal.

In summarizing the work to date from this laboratory with regard to the influence of autoclaving soybean oil meal on the nutritive value and digestibility of the protein, the data presented in table 2 are of interest. A comparison of the

protein efficiency, as measured by the grams gain in weight of growing chicks during a 4-week period divided by the grams of protein consumed during this period, and the amino groups liberated by *in vitro* trypsin and erepsin digestion of the soybean oil meal showed a coefficient of correlation of + 0.903. One of 0.811 was required for significance. A coefficient of correlation of + 0.769 which was not significant was obtained between protein efficiency and amino groups liberated by *in vitro* pepsin, trypsin, and erepsin digestion. These values indicate that autoclaving affects soybean oil meal in the same way for liberation of amino groups by trypsin and erepsin *in vitro* and for efficiency of chick protein utilization. It would appear from these results and those presented in table 1 that the major part of the digestive activity was characteristic of a trypsin and erepsin system. This may at least partially explain why attempts by various workers in the past have not shown a relation between *in vitro* pepsin digestion and true digestibility or biological value for soybean oil meals. Evans ('45a) observed no relation between the pepsin digestible sulfur of commercial soybean oil meals and their protein nutritive value for chicks.

The per cent glutelin in 10 commercial soybean oil meals was shown by Evans and St. John ('45) to have a highly significant correlation with the protein nutritive values as measured by the gross protein value determined by the procedure of Heiman, Carver and Cook ('39) when no uncooked soybean oil meal was included in the calculation. The values for per cent of protein measured as glutelin in the soybean oil meals studied in this experiment are presented in table 2. The highest glutelin value was in the meal autoclaved for 30 minutes at 110°C. Autoclaving increased the per cent protein measured as glutelin (soluble in 0.2% KOH solution but insoluble in water, 5.0% KCl solution or 70% alcohol) over that in the raw soybean oil meals except for the meals autoclaved at 130°C. for 30 or 60 minutes which contained less protein determined as glutelin than the raw meal. Excluding the raw soybean oil meal from the calculations, a coefficient of

correlation of + 0.887 between per cent glutelin and protein efficiency was obtained. A value of 0.878 was required for significance. Including the raw soybean oil meal in the calculations, a coefficient of correlation of + 0.854 was obtained. One of 0.811 was required for significance.

SUMMARY

Chicks were raised to 4 weeks of age on diets supplemented with raw soybean oil meals which had received the following treatments: none, autoclaved for 30 minutes at 100, 110, 120, 130°C. or autoclaved for 60 minutes at 130°C. The percentages of total protein, organic sulfur, cystine and methionine in the soybean oil meals not digested by the chick, by pepsin, trypsin and erepsin *in vitro,* or by trypsin and erepsin *in vitro* were determined. The soybean oil meals which had been autoclaved at temperatures between 100°C. and 120°C. for 30 minutes were more completely digested by the chick or by trypsin and erepsin *in vitro* than the raw meal or the meals which had been autoclaved at 130°C. using undigested total protein, or sulfur, cystine, or methionine in the undigested protein as criteria. Raw soybean oil meal was in most cases more readily digested by pepsin, trypsin and erepsin *in vitro* than the autoclaved meals. Significant correlations were obtained between *in vitro* trypsin and erepsin digestion, and chick digestion of these soybean oil meals for undigested protein, organic sulfur and cystine. No significant correlations between chick digestion and *in vitro* pepsin, trypsin and erepsin digestion values were obtained. Of especial interest is the relatively high percentage of cystine in the soybean oil meals that was not digested by the chick or by either of the *in vitro* enzyme digestions made.

LITERATURE CITED

ALMQUIST, H. J., E. MECCHI, F. H. KRATZER AND C. R. GRAU 1942 Soybean protein as a source of amino acids for the chick. J. Nutrition, *24:* 385.

ANSON, M. L. 1945 Protein denaturation and the properties of protein groups. Advances in Protein Chem., *2:* 361.

ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS 1945 Official Methods of Analysis, par. 2.26, p. 27.

BOWMAN, D. E. 1944 Fractions derived from soybeans and navy beans which retard tryptic digestion of casein. Proc. Soc. Exp. Biol. Med., *57:* 139.

CARVER, J. S., J. MCGINNIS, C. F. MCCLARY AND R. J. EVANS 1946 The utilization of raw and heat-treated soybean oil meal for egg production and hatchability. Poultry Sci., *25:* 399.

EVANS, R. J. 1945a The sulfur content of the pepsin digestible fraction of protein concentrates. Arch. Biochem., *7:* 33.

———— 1945b Determination of the cystine and methionine content of plant and animal material by a differential oxidation procedure. Arch. Biochem., *7:* 439.

———— 1946 Hydrolysis of soybean oil meal proteins by some proteolytic enzymes. Arch. Biochem., *11:* 15.

EVANS, R. J., AND J. E. GREAVES 1937 Some factors influencing the sulfur content of alfalfa. Proc. Utah Acad. Sci., *14:* 17.

EVANS, R. J., AND J. MCGINNIS 1946 The influence of autoclaving soybean oil meal on the availability of cystine and methionine for the chick. J. Nutrition, *31:* 449.

EVANS, R. J., AND J. L. ST. JOHN 1944 Determination of total sulfur in feeds. Modified nitric and perchloric acid digestion procedure. Ind. Eng. Chem., Anal. Ed., *16:* 630.

———— 1945 Estimation of the relative nutritive value of vegetable proteins by 2 chemical methods. J. Nutrition, *30:* 209.

HAM, W. E., AND R. M. SANDSTEDT 1944 A proteolytic inhibiting substance in the extract from unheated soybean meal. J. Biol. Chem., *154:* 505.

HAM, W. E., R. M. SANDSTEDT AND F. E. MUSSEHL 1945 The proteolytic inhibiting substance in the extract from unheated soybean oil meal and its effect upon growth in chicks. J. Biol. Chem., *161:* 635.

HEIMAN, V., J. S. CARVER AND J. W. COOK 1939 A method for determining the gross value of protein concentrates. Poultry Sci., *18:* 464.

JOHNSON, L. M., H. T. PARSONS AND H. STEENBOCK 1939 Effect of heat and solvents on the nutritive value of soybean protein. J. Nutrition, *18:* 423.

KUNITZ, M. 1945 Crystallization of a trypsin inhibitor from soybean. Science, *101:* 668.

LUND, A. P., AND W. M. SANDSTROM 1943 The proteins of various tree seeds. J. Agr. Research, *66:* 349.

MCGINNIS, J., AND V. H. MENZIES 1946 Effect of in vitro enzymatic digestion of raw soybean flakes on chick growth. Poultry Sci., *25:* 538.

MELNICK, D., B. L. OSER AND S. WEISS 1946 Rate of enzymic digestion of proteins as a factor in nutrition. Science, *103:* 326.

SCHOENING, H. W. 1939 The digestive processes in domestic animals. Food and Life, Yearbook of Agric., U. S. Dept. of Agric., p. 425.

ST. JOHN, J. L., AND O. JOHNSON 1931 The determination of uric acid in the study of avian nutrition. J. Biol. Chem., *92:* 41.

NUTRITIVE VALUE OF KERATIN

III. EFFECT OF SOURCE, PARTICLE SIZE, AND METHOD OF GRINDING [1]

G. W. NEWELL AND C. A. ELVEHJEM
Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison

(Received for publication December 30, 1946)

INTRODUCTION

Keratin is generally defined as a protein insoluble in dilute acid or alkali, and resistant to digestion by the common proteolytic enzymes. Meunier et al. ('27) however, showed that when wool is digested with alkali at pH 9–10, it becomes partially susceptible to the proteolytic action of pancreatin, and Routh and Lewis ('38) demonstrated an even greater digestion of wool by trypsin and pepsin after grinding in a ball mill.

Recently, reports have come from several laboratories concerning the nutritional value of powdered keratins. Routh ('42) used powdered wool, and later ('42a) powdered chicken feathers as the only source of protein in purified rations for rats. Growth response, although poor, was most marked when amino acid supplements of lysine, histidine, methionine and

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Supported in part by a grant from Wilson and Co., Chicago, Ill.

We are indebted to the Wilson Laboratories, Chicago, Ill., for the liver products; to Wilson and Company, Chicago, Ill., for some of the keratin samples; to Merck and Company, Rahway, N. J., for the synthetic vitamins; and to the Abbott Laboratories, North Chicago, Ill., for the supply of Haliver Oil.

tryptophane were included in the rations. Wagner and Elvehjem ('43) found that powdered hog hoofs could be substituted for meat-scrap or fishmeal supplements in practical poultry rations. In the nutritional experiments cited the keratins were prepared by grinding in a ball mill for prolonged periods of time.

In this paper we wish to describe the growth of rats and chicks fed purified diets in which the protein was, in whole, or in part, keratin prepared by different methods. The effect of amino acid supplements on the growth of rats receiving certain of these keratin preparations is also presented.

EXPERIMENTAL

Two-day old chicks obtained from commercial hatcheries, 6 per group, were kept on raised wire screens in electrically heated cages. Weanling male rats, of the Sprague-Dawley strain, weighing 40 to 50 gm were kept in individual raised wire cages. Rations and water were fed *ad libitum* throughout the 4-week experimental period. The composition of the rations used is given in tables 1 and 3. In all cases the level of protein was varied at the expense of sucrose.

Keratins used in this study were hog hoofs, summer hog hair, and chicken feathers. These proteins were prepared by powdering in a ball mill for 3 to 7 days. Several samples of hoof keratins were subjected to different types of grinding procedures. Keratin-A was powdered in a ball mill, while keratin-B was ground in a hammer mill and then finally powdered in a Wiley mill. In both these methods considerable heat is developed during the grinding procedure. The third hoof sample, keratin-C, was prepared by a process which rapidly pulverizes the keratin to a fine powder without heating. All keratins were ground fine enough to pass an 80 mesh screen except in the experiment where particle size was studied. Special effort was made to determine which amino acids were necessary to supplement the powdered hoofs (18 and 24% levels) in order to obtain growth comparable to that of an equal level of casein.

RESULTS

Powdered keratin as a protein source in chick rations

The diets used for the chicks and the growth responses obtained are given in table 1. Group 1, which received casein and cartilage serves as the reference group. The growth obtained is representative of that produced by purified rations

TABLE 1

Effect of ball mill powdered keratins on growth and survival of chicks. (6 chicks per group.)

GROUP NO.	1	2	3	4	5	6	7	8	9	10	11
Casein [1]	18	18	10					10			18
Cartilage	10										
Powdered hog hair		10	18	24	30	30	30				
Powdered hog hoofs (keratin-A)								18	24	30	
Powdered chicken feathers											10
Powdered oat meal							5				
α-tocopherol mg/day/chick						1					
Sucrose	54	54	54	58	52	52	47	54	58	52	54
Average weight at 4 weeks	244	216	212	94	116	57	59	227	157	216	132
Encephalomalacia	—	+	+	+	+	—	—		—	—	—
Gizzard erosion											
Slight	3						4		1	1	3
Moderate	3	6	2	6		6	2		2	4	
Severe			4		3				3	1	3
Number dead at 4 weeks		1	2	1	3						

[1] All rations contained in addition to the above listed constituents: brewer's yeast, 5%; soybean oil, 5%; Salts IV, 4%; solubilized liver powder, 2%; cod liver oil, 2%; and vitamin supplements per kilo of ration as follows: thiamine hydrochloride, 3 mg; riboflavin, 3 mg; pyridoxine hydrochloride, 3 mg; calcium pantothenate, 15 mg; choline hydrochloride, 1.5 gm.

of this type. Powdered hog hair at levels of 24% or 30% of the ration produced poor growth (groups 4 and 5). Feathering of chicks fed this protein was only fair. High levels of powdered hog hair tended to cause the occurrence of nutritional encephalomalacia. When casein was combined with

hog hair in a ration at 18% and 10% (group 2), or at 10% and 18% (group 3), respectively, good growth was obtained. Encephalomalacia still occurred with these protein combinations but the incidence decreased as the percentage of powdered hair was reduced. Pappenheimer et al. ('39) were able to prevent the incidence of encephalomalacia in chicks fed purified rations by oral administration of small quantities of α-tocopherol. Two additional groups were fed 30% powdered hair; 1 group received oral supplements of 1 mg α-tocopherol per chick per day, and the other group was fed 5% powdered oats as a natural source of vitamin E. None of the chicks in these 2 groups exhibited any signs of encephalomalacia. The growth rate was only about half that obtained in group 5 but the hair used for groups 6 and 7 was ground for 6 days and this long grinding may have been responsible for the reduced growth. Group 11 included 10% chicken feathers and 18% casein as the protein source. Growth was only about half that obtained in the reference group even though the level of casein was not reduced. Feathering of chicks on this ration was very poor.

Poor growth resulted when powdered hoofs were fed at a 24% level (group 9) but when the level was increased to 30% (group 10), good growth was obtained. When 18% of powdered hoofs was combined with 10% of casein, the response obtained closely approximated the maximum growth of chicks fed the casein-cartilage reference ration. At all levels of hog hoofs used in these studies, good feathering was observed.

The gizzards of chicks fed the above-mentioned keratins were examined for erosion. Gizzards were graded according to the severity of the lesions as slight, moderate, or severe. It was not possible to use complete prevention of gizzard erosion as a criterion, since chicks under 6 weeks of age rarely have gizzards entirely free of lesions. Powdered hog hair consistently caused moderate to severe erosion of the gizzard. The majority of the fissures found were parallel to the normal folds of the linings. Chicken feathers were variable in their effect, but the severity of the lesions consistently fol-

lowed the poor feathering of the birds fed this protein. Gizzards of chicks fed powdered hog hoofs were comparatively free of erosion and compared favorably with the gizzards of chicks fed the reference ration.

During the course of these studies, a question arose as to what role the particle size of powdered keratin might play in the growth of chicks. It had been noticed that when keratins were powdered for long periods in a ball mill, the nutritive quality of the protein was materially decreased. An experiment was carried out in which powdered hog hoofs which passed screens with mesh sizes of 10, 20, 40, 60 and 80 were included in the ration at a level of 30% (table 2). One series

TABLE 2

Effect of different methods of grinding keratin on the growth of chicks[1] *(gm gained in 4 wks.; 6 chicks per group).*

MESH SIZE	10	20	40	60	80
Hog hoofs powdered in ball mill (keratin-A)	..	96	60	88	125
Hog hoofs ground successively in hammer and Wiley mills (keratin-B)	134	141	140	158	160

[1] Hog hoofs at 30% of ration constituted the sole protein.

of chicks received hoofs powdered in a ball mill (keratin-A) while the second series was fed powdered hoofs prepared in a hammer and a Wiley mill (keratin-B). That destructive action takes place in the ball mill is clearly demonstrated by the poor growth of chicks fed this product, regardless of the particle size. Hoofs powdered in the Wiley mill gave the best results when chicks were fed the material passing the 80 mesh screen. A gradual decrease in growth response was noted as the particle size was increased.

Powdered keratins as a source of protein for the growing rat

Results obtained when rats were fed rations in which keratin was used as the source of protein are given in table 3.

A group without any protein (R–I) and one given 18% casein (R–II) are included for reference.

When powdered hog hair was fed at a level of 18% of the ration (R–V) the rats showed a growth of only 6 gm in 4 weeks. The animals fed rations in which casein replaced one-third (R–IV) or one-half (R–III) of the hair showed greatly increased growth, although the response did not approach the rate obtained with animals which received 18% of casein.

TABLE 3

Effect of powdered keratins (ball milled) on growth of rats. (6 rats per group.)

RATION (R)[1]	I	II	III	IV	V	VI	VII	VIII	IX
Casein		18	9	6		9	6		
Powdered hog hair			9	12	18				
Powdered hog hoofs						9	12	18	24
Sucrose	89	71	71	71	71	71	71	71	71
Gm gained in 4 weeks	— 15	139	109	101	6	133	123	55	60

[1] All diets contained in addition to the above listed constituents: Salts IV, 4%; corn oil, 5%; liver powder 1–20, 2%; and vitamin supplements per kilo of ration as follows: thiamine hydrochloride, 2 mg; riboflavin, 1 mg; pyridoxine hydrochloride, 3 mg; calcium pantothenate, 20 mg; choline chloride, 1 gm. Each rat received 1 drop of Haliver Oil per week.

When hoofs powdered in a ball mill were used as the sole source of protein at a level of 18% in the ration (R–VIII), a growth response of 55 gm was obtained in 4 weeks. The rate of growth is poor as compared with that obtained with 18% of casein but markedly better than that obtained with the same level of powdered hair. When the level of powdered hoofs was increased to 24% of the ration (R–IX) little improvement in growth resulted. With protein at 18% of the ration, substitution of one-third of the powdered hoofs with casein (R–VII) resulted in moderate growth, while a combination of 9% of hoofs and 9% of casein, (R–VI), produced growth approaching that obtained with 18% of casein.

Effect of method of preparation and of amino acid supplements on the growth of rats fed powdered hog hoofs

In these studies the rations were patterned after those described in table 3. Groups 1 and 2 receiving 18% and 24% of casein (lines 1 and 2, table 4), served as optimum reference groups.

TABLE 4

Growth response of rats to hog hoofs prepared by different methods. (3 rats per group.)

	RATION AND SUPPLEMENTS	GM GAINED IN 4 WEEKS ON:	
		Keratin-A [1]	Keratin-C [2]
1	18% Casein	134	
2	24% Casein	143	
3	18% Hog hoofs (R VIII)	40	66
4	24% Hog hoofs (R IX)	42	112
5	30% Hog hoofs	112	130
6	40% Hog hoofs		140
7	R VIII plus 0.6% lysine, 0.1% tryptophane, 0.3% methionine, 0.6% cystine	27	
8	R VIII plus 0.6% lysine, 0.3% methionine, 0.6% cystine, 0.4% histidine	95	
9	R VIII plus 0.1% tryptophane, 0.3% methionine, 0.6% cystine, 0.4% histidine	91	
10	R VIII plus 0.6% lysine, 0.1% tryptophane, 0.3% methionine, 0.4% histidine	109	124
11	R IX plus amino acids as in 10		136
12	R VIII plus 0.6% lysine, 0.2% tryptophane, 0.3% methionine, 0.4% histidine		121
13	R IX plus amino acids as in 12		128
14	R VIII plus 0.6% lysine, 0.2% tryptophane, 0.6% methionine, 0.4% histidine		121
15	R IX plus amino acids as in 14		136
16	R IX plus 0.6% lysine, 0.2% tryptophane, 0.6% methionine, 0.4% histidine, 0.4% phenylalanine		137
17	R IX plus 0.6% lysine, 0.05% tryptophane, 0.3% methionine, 0.3% histidine, 0.3% phenylalanine		118

[1] Powered in a ball mill.

[2] Powdered with little or no heat production.

Data from the chick experiments indicate that there is a difference in the nutritive quality of keratins, dependent upon the method of preparation. As in the earlier experiments, growth of rats fed hoofs at an 18% level was poor. However, keratin-C (powdered with no heat production) gave a 55% better growth response than did keratin-A (powdered in a ball mill). When the level of hoofs was raised to 24% a more marked difference between the 2 keratin preparations could be noted. When keratin-C was included in the ration at 30%, a growth rate which approximated that obtained with 18% casein was obtained. Only rather moderate growth resulted when rats were fed keratin-A at this level. At 40% of the ration keratin-C produced growth comparable to that obtained with 24% casein. The hair coat of animals fed powdered hog hoofs was always heavy and glossy. At no time was there any indication of toxicity due to the feeding of high levels of keratin.

The necessity of feeding such high levels of keratin (30% to 40%) suggested that certain essential amino acids are probably present in limited amounts. This view is substantiated by the data of Routh ('42, '42a) which indicate that tryptophane, methionine, lysine and histidine are necessary supplements to diets where powdered wool or feathers supply the protein. In this laboratory amino acid supplements of 0.6% lysine, 0.1% tryptophane, 0.3% methionine and 0.6% cystine to ration R–VIII (line 7, table 4) produced a decrease in growth as compared with that obtained with 18% of keratin-A alone. Substitution of 0.4% histidine for the tryptophane produced a marked improvement of growth. The combination of tryptophane, methionine, cystine and histidine resulted in the same rate of growth. Elimination of cystine and use of the 4 amino acids, lysine, tryptophane, methionine and histidine, found most efficient by Routh in supplementing wool protein, gave the best growth. When keratin-C was increased to 24%, and the same amino acids used as before (line 11), young rats grew at a rate comparable to that produced by 18% of casein. No improvement was obtained with

R–VIII when the tryptophane (line 12) or the methionine (line 14) supplements were increased 100%. When these increased amino acid supplements were added to R–IX (lines 13 and 15), growth as good as that demonstrated in line 11 was obtained. Microbiological assay of keratin-C suggested that the amount of phenylalanine present was at a critical level if the protein were fed at 18% or even 24% of the ration. However, when 0.4% phenylalanine was added to R–IX, in addition to methionine, tryptophane, lysine and histidine (line 16) growth was the same as that obtained in line 11.

After examination of the amino acid values tabulated by Block and Bolling ('45) for horn keratins, calculations were made to determine the level of essential amino acids furnished by powdered hoofs when fed at 24% of the ration. These estimates suggested that if supplements of 0.6% lysine, 0.05% tryptophane, 0.3% methionine, 0.3% histidine and 0.3% phenylalanine were made to R–IX, a growth response comparable to that of 18% casein would be obtained. This expected response was not achieved since growth (line 17) was only slightly better than when unsupplemented R–IX was fed (line 4). These results may indicate that the portion of the amino acids supplied by powdered hoofs was only partially available.

DISCUSSION

The data presented indicate that rats and chicks are able to utilize powdered keratins although the origin and method of preparation markedly affect the biological value of these proteins. Hoofs gave the best growth response in both rats and chicks, while hair was definitely inferior. Feathers appeared to be the least suitable nutritional material of the 3 keratins tried.

The preparation of this type of protein also greatly influences its nutritional value. Data on the effect of particle size on the growth of chicks are presented and similar results, not tabulated, were obtained with rats. Not only must the product be finely divided but the method of bringing about

this breakdown is very important. Fineness of division appears necessary to speed enzymatic hydrolysis and prolonged grinding appears to produce nutritionally undesirable changes, possibly as a result of heating. Routh and Lewis ('38) have shown that inorganic sulfates not originally present in wool are produced by the prolonged ball mill grinding, and they suggest that these are oxidation products. Hog hoof keratin was the most intensively investigated but if methods are developed to reduce hair or feathers to sufficient fineness without producing undesirable changes, these keratins might become more valuable.

Ball mill grinding appears to be destructive from both mechanical and chemical standpoints. Briggs et al. ('42) have shown that erosion of the gizzard may occur when there is an insufficient supply of glycine, arginine, and cystine in the chick ration. The severity of gizzard erosion in chicks which received hair would imply that the amino acids present in adequate amounts in the original hair were not available to the chick. When fed powdered hair numerous chicks developed encephalomalacia which was cured to α-tocopherol. Why this condition developed on powdered hair and not on other keratins is not clear.

Substantial growth was obtained when ball milled hog hoofs were fed to rats and chicks at 30% of the ration. The specially ground hoofs at this level supported growth equal to that of 18% of casein. With supplements of lysine, tryptophane, methionine, and histidine, 18% of hog hoofs gave growth which also approximated that obtained with casein. Data, not tabulated here, have established that omission of any one of the 4 amino acids results in growth no better than that obtained without supplementation.

Because of the physical changes brought about when keratins are powdered, the practical application of new protein sources should not be overlooked. This work confirms the suggestions of Routh ('42) and Wagner and Elvehjem ('42), and indicates that careful preparation of keratinous material

offers a means whereby waste protein of high nutritive quality may be adapted for use in animal feeds.

SUMMARY

Finely powdered keratins have been studied as possible protein sources for growing rats and chicks. When chicks or rats were fed purified rations which contained 30–40% of powdered hoofs, substantial growth was obtained. High levels of powdered hog hair in chick rations allowed moderate growth but usually produced an encephalomalacia unless sources of vitamin E were present. Powdered chicken feathers allowed only poor growth.

In general, the rates of growth obtained with chicks and rats fed rations containing these keratins show a positive correlation with the degree of subdivision of the keratin. The use of a ball mill for grinding the keratin was not as effective a method as one in which heating was prevented. When the lower levels of powdered hoofs were supplemented with lysine, tryptophane, methionine, and histidine, good growth was obtained.

LITERATURE CITED

BLOCK, R. J., AND D. BOLLING 1945 The Amino Acid Composition of Proteins and Foods. 1st ed. Charles C. Thomas, Springfield.

BRIGGS, G. M., JR., R. C. MILLS, C. A. ELVEHJEM AND E. B. HART 1942 The effect of added cystine in purified rations for the chick. J. Biol. Chem., *144:* 47.

MEUNIER, L., P. CHAMBARD AND H. COMTE 1927 Sur la digestion pancréatique de la laine. Compt. rendu des Societie de L'academie des Sciences, *184:* 1208.

PAPPENHEIMER, A. M., M. GOETTSCH AND E. JUNGHERR 1939 Nutritional encephalomalacia in chicks. Conn. Exp. Sta. (Storrs) Bull. 229, p. 121.

ROUTH, J. I. 1942 Nutritional studies on powdered wool. J. Nutrition, *23:* 125.

——— 1942a Nutritional studies on powdered chicken feathers. J. Nutrition, *24:* 399.

ROUTH, J. I., AND H. B. LEWIS 1938 The enzymatic digestion of wool. J. Biol. Chem., *124:* 725.

WAGNER, J. R., AND C. A. ELVEHJEM 1942 The nutritive value of keratins. I. Proc. Soc. Exp. Biol. Med., *51:* 394.

——— 1943 The nutritive value of keratins. II. Poultry Sci., *22:* 275.

A STUDY OF THE RELATION BETWEEN PROTEIN EFFICIENCY AND GAIN IN WEIGHT ON DIETS OF CONSTANT PROTEIN CONTENT [1]

D. M. HEGSTED AND JANE WORCESTER

Departments of Nutrition, Biological Chemistry, and Biostatistics, Harvard School of Public Health and Harvard Medical School, Boston

TEN FIGURES

(Received for publication January 17, 1947)

Osborne, Mendel and Ferry ('19) originally proposed the use of "grams gain per gram of protein eaten" (protein efficiency) as a measure of the nutritive value of proteins for growing rats. This index combined the 2 variables of food intake and gain in weight into a single figure and thus was thought to be a superior measure of nutritive value than the simple measurement of either variable alone, that is, gain in weight or food intake. In subsequent years, protein efficiency has gained wide acceptance as a measure of the nutritive value of proteins.

In the original paper, and subsequently, it was clearly shown that the level of the protein in the diet affects the value obtained for the protein efficiency. With increasing levels of protein, it reaches a maximum and then slowly declines. Osborne et al. proposed that the level of maximum efficiency be determined for each protein. In practice, however, most authors including the originators fed various proteins at arbitrary levels and compared the protein efficiency values thus obtained.

[1] Supported in part by a grant-in-aid from The American Meat Institute, Chicago, Ill.

Mitchell ('24, '42) has criticized this method on various scores, principally on the basis that the composition of the body gains may not be constant and that no credit is given to the protein for maintenance. He has observed (Mitchell, '24) that in general animals with larger food intakes tend to show the highest protein efficiency "although the correlation is not particularly close."

It is remarkable in view of the wide acceptance of the method as a measure of the nutritive value of proteins that the relation between gain in weight and protein efficiency appears not to have been carefully analyzed. In studies on the value of various supplements to white flour conducted in this laboratory, the calculation of protein efficiency apparently added no additional information over the simple measure of body weight. These studies and an additional experiment designed to study the relation of protein efficiency to gain in weight are reported in this paper.

EXPERIMENTAL

All of the studies were made with young male rats obtained from the same breeder. Their average weight at the time the various studies were started was approximately 45 gm. Groups of 5 or 6 rats were fed each ration ad libitum. The white flour was always of the same brand but 2 lots were used which contained 13.3% and 14.4% protein (N $\times$ 6.25). The protein supplements were commercial products.

All of the diets contained 12% protein (N $\times$ 6.25). In the studies on white flour, the protein was supplied by appropriate amounts of the supplement and flour. Mixtures of casein and wheat gluten supplied the protein in the other experiment. Salt mixture 4% (Hegsted et al., '41), K_2HPO_4, 0.5%, corn oil 4% [2] were always added and the ration completed with sucrose. Crystalline thiamine, riboflavin, pyridoxine, calcium pantothenate, nicotinic acid, and choline chloride [3] were added to all rations in the amounts reported previously (Hegsted

[2] Supplied by the Corn Industries Research Foundation, New York, N. Y.
[3] Supplied by Merck and Co., Rahway, N. J.

et al., '44), and 1 drop of haliver oil containing viosterol was given twice weekly to each rat. Each experiment was continued for 3 weeks.

Food consumption records were made by weighing the amount of food given and the amount left or spilled each day. Small food cups containing the ration were placed inside of larger dishes and papers underneath the cages recovered any spilled ration.

RESULTS

Figure 1 shows the average gain in weight and protein efficiency of the group of animals which received white flour alone or white flour and varying amounts of skim milk powder,

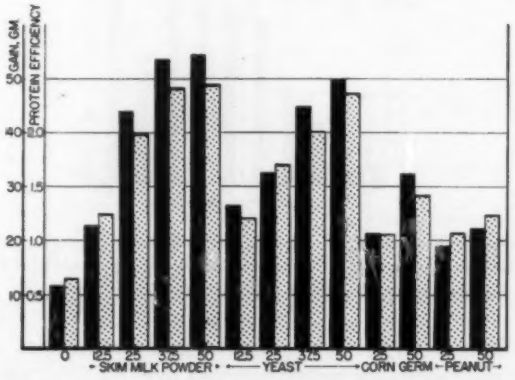


Fig. 1 Average gain in weight and protein efficiency of the groups of rats receiving white flour and white flour plus supplements. The figures on the abscissa are the per cent of the total protein supplied by the supplement. Solid bars represent gain in weight.

yeast, corn germ, and peanut flour. The differences in the supplemental value of the various proteins are apparent. Moreover, it should be noted that the proteins of the different diets are classified, relative to each other, in the same order whether protein efficiency or gain in weight is used as the measure of nutritive value.

Figure 2 is a similar chart of the data obtained using 10 diets in which the nutritive value was varied by the relative proportions of gluten and casein. This experiment was designed to give a series of proteins of gradually increasing nutritive value. It may be noted in comparing figures 1 and 2 that the protein efficiency is somewhat higher relative to the gain in weight in this experiment than in the first experiment. However, the chief point of interest is that the protein mixtures are again classified in the same relative position by either gain in weight or protein efficiency.

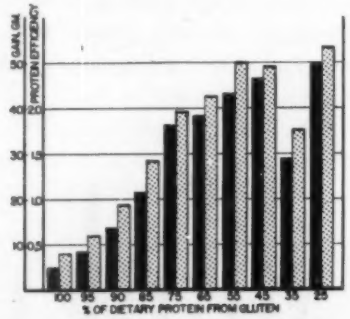


Fig. 2 Average gain in weight and protein efficiency of the groups receiving mixtures of gluten and casein. The remainder of the total protein (12% of the ration) was supplied by casein. Solid bars represent gain in weight.

This is true even for group 9 which is anomalous due to the inclusion of 2 animals which grew very poorly. One animal in this group died on the second day. The data are thus based on only 4 animals, 2 of which appeared abnormal. It seemed worthwhile, therefore, to investigate the relation between the protein efficiency and gain in weight of the individual animals.

Figure 3 is a scatter diagram showing the relation of protein efficiency to gain in weight for each of the rats in the first experiment. The straight line (no. 1) is the regression line fitted by the method of least squares. This fits the data reasonably well and the correlation coefficient is + 0.97. However, the regression line appears to fall above the experimental

points at both extremes of the data, the variation about the line increases with increases in gain in weight, and finally the line fails to pass through the origin of the graph although by definition protein efficiency is zero when the gain is zero.

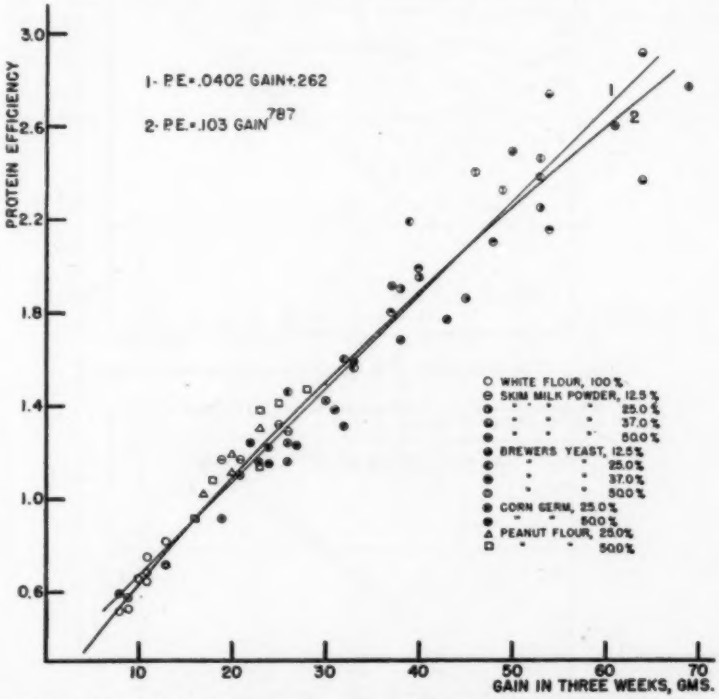


Fig. 3 The relation between the protein efficiency and gain in weight of the individual rats in Experiment I.

These difficulties disappear when the data are plotted as logarithms in figure 4.[4] The correlation is increased to + 0.984 and the data fall uniformly about the line.

The regression line log 10 P.E. = 0.787 log gain + 0.0128 may be put in terms of the original data to give the equation

[4] Protein efficiency times 10 (10 P.E.) is used rather than the original protein efficiencies for ease in plotting.

P.E. $= 0.103$ gain $^{0.787}$. The curve of this equation is shown in figure 3 as no. 2. This line fits the data at the 2 extremes better than the previous line and also approaches the origin.

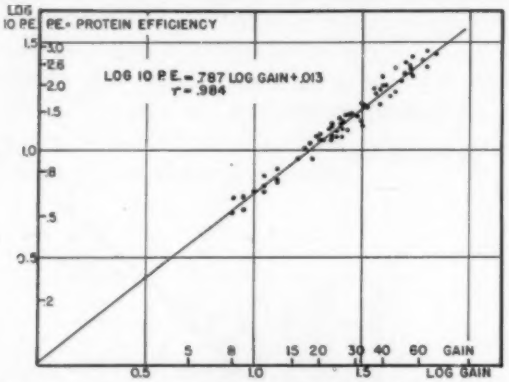


Fig. 4 The same data as in figure 3 plotted as logarithms.

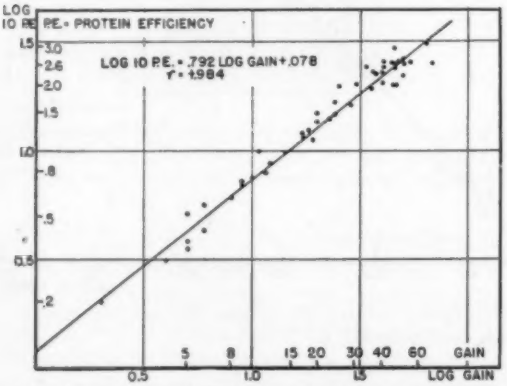


Fig. 5 A scatter diagram showing the protein efficiencies and gains in weight for the individual rats in Experiment II. The data are plotted as logarithms.

Figure 5 represents a log-log plot of the data from Experiment II. Again the data clearly fall about a straight line and the coefficient of correlation is $+ 0.984$. When this line is put in terms of the original data, the equation P.E. $= 0.0834$ gain $^{0.792}$ is obtained which is shown in figure 6.

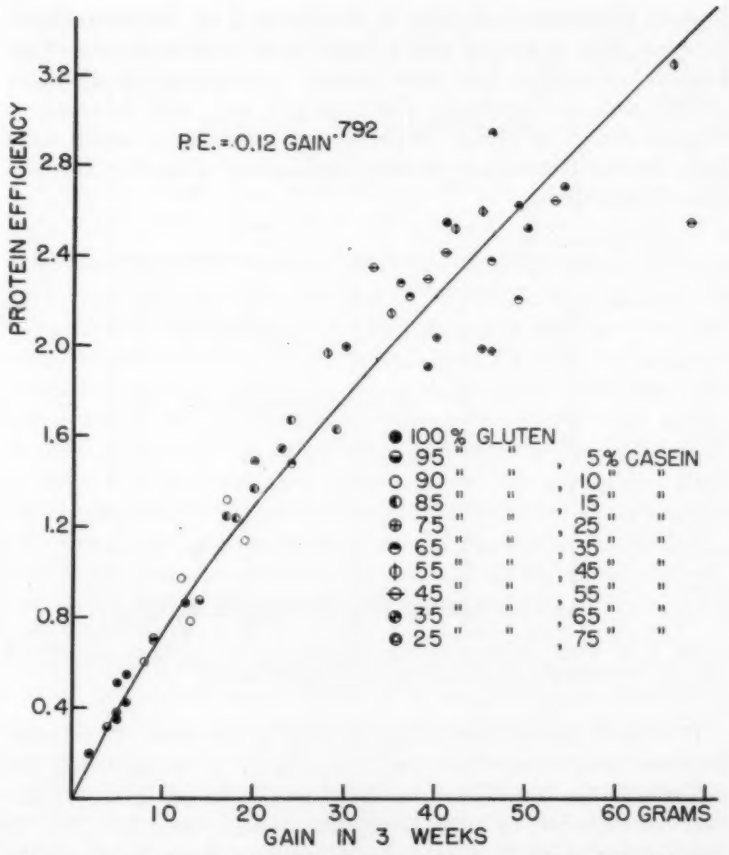


Fig. 6 The relation between protein efficiency and gain in weight in Experiment II.

DISCUSSION

In these experiments the gain in weight, amount of protein eaten and protein efficiency are known for each rat. The first 2 variables, gain in weight and the amount of protein eaten, are independent in the sense that they can be measured independently on each rat while the third variable, protein efficiency, is the ratio between the 2. The correlation between

protein efficiency and gain in weight is thus the correlation between gain in weight and a function of gain in weight. This kind of correlation has been termed "spurious" by Pearson (1897) but, as Snedecor ('46) points out, this derogatory remark would be better reserved for conclusions which have been drawn from such correlations rather than the correlations themselves.

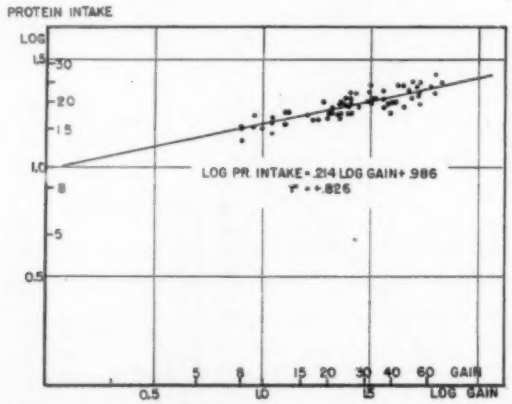


Fig. 7 A scatter diagram showing the relation of the logarithms of food intake to the logarithms of weight gains in Experiment I.

It is well known that there is in general some correlation between food intake and gain in weight. Even allowing for differences in nutritive value, animals which grow faster may be expected to have somewhat greater food intakes. In the present work, this is demonstrated in figure 7 where the logarithms of the values for protein intake in Experiment I have been plotted against the logarithms of the gains in weight. The data appear to be well represented by the regression line:

$$\log \text{protein intake} = 0.214 \log \text{gain} + 0.986.$$

However, the correlation coefficient is only + 0.826 compared to the very high correlation between protein efficiency and gain in weight.

This discussion is pertinent to the present problem since the regression lines in figures 2 and 4 might be solved in the following manner:

$$\text{Log 10 P.E.} = 0.787 \log \text{gain} + 0.013 \tag{1}$$

$$\text{Since log P.E.} = \log \frac{\text{gain}}{\text{protein intake}} = \log \text{gain} - \log \text{protein intake}$$
$$\text{then log gain} - \log \text{protein intake} + 1 = 0.787 \text{ gain} + 0.013 \tag{2}$$

$$\text{and log protein intake} = 0.213 \log \text{gain} + 0.987 \tag{3}$$

By such reasoning one thus arrives at the conclusion that the protein intake, regardless of nutritive value, is predicted by the gain in weight. However, equation 3 is the same as the equation for the regression line in figure 7 and it has already been shown by the coefficient of correlation, + 0.826, that this line predicts the protein intake only with considerable error. In fact, this coefficient of correlation indicates that only 68% of the variation has been explained by the regression line. This example thus demonstrates the erroneous conclusion which might be arrived at because of "spurious" correlation. It is clear that the excellent correlation between protein efficiency and gain in weight does not necessarily imply an equally good correlation between protein intake and gain in weight.

As has been discussed by numerous other authors, the concept of protein efficiency suffers serious theoretical difficulties. Dietary protein must be considered as fulfilling 2 functions (a) maintenance and (b) growth. Since the calculation of protein efficiency does not consider the protein required for maintenance, considerable improvement at least theoretically would result, if the maintenance protein requirement could be subtracted from the protein intake, and only the remainder considered in the calculation of protein efficiency. Boas-Fixen et al. ('34) attempted this and estimated the maintenance requirement by extending the regression line of protein intake vs. gain to the intercept of zero gain. It is readily apparent, however, that this may not be a valid procedure since undoubtedly the animals which grow have larger maintenance requirements due to the increase in body size. Furthermore, maintenance requirements cannot be estimated from the body

size since the proteins vary in nutritive value, and the maintenance requirement per unit of body size is less for the better proteins. Thus the maintenance requirement for the animals which grow (those receiving proteins of higher nutritive value) tends to be higher because of the increase in size while at the same time must be less per unit of body size than for animals which do not grow because of the difference in the nutritive value of the proteins they receive. Whether it is actually higher or lower or relatively constant for animals of varying gains will require further investigation and no satisfactory method of estimating it appears to be available at the

TABLE 1

The relative rank given the 10 proteins in Experiment II by the 3 methods of classification.

PROTEIN	AMOUNT EATEN	GAIN IN WEIGHT	PROTEIN EFFICIENCY
1	1	1	1
2	2	2	2
3	3	3	3
4	4	4	4
5	8	6	6
6	7	7	7
7	6	8	8
8	10	9	9
9	5	5	5
10	9	10	10

present time. We must, therefore, consider protein efficiency as simply a method of classifying proteins *relative to each other* as to their nutritional value. The close correlation between gain in weight and protein efficiency indicates that either of these will classify proteins as to their nutritive value in a similar manner. Since protein intake alone shows a correlation with gain in weight, it might also be used to assess the nutritive value of proteins. Table 1 shows the order in which the 10 proteins fed in Experiment II ranked according to the 3 methods. There is perfect agreement by either protein efficiency or gain in weight, but food intake alone fails to dis-

tinguish between the last 5 proteins. The same close correlation between protein efficiency and gain in weight is shown by data in the literature. As examples the data from the 2 papers by Jones and coworkers have been plotted in figure 8 as logarithms. In the first paper (Jones and Divine, '44) 16

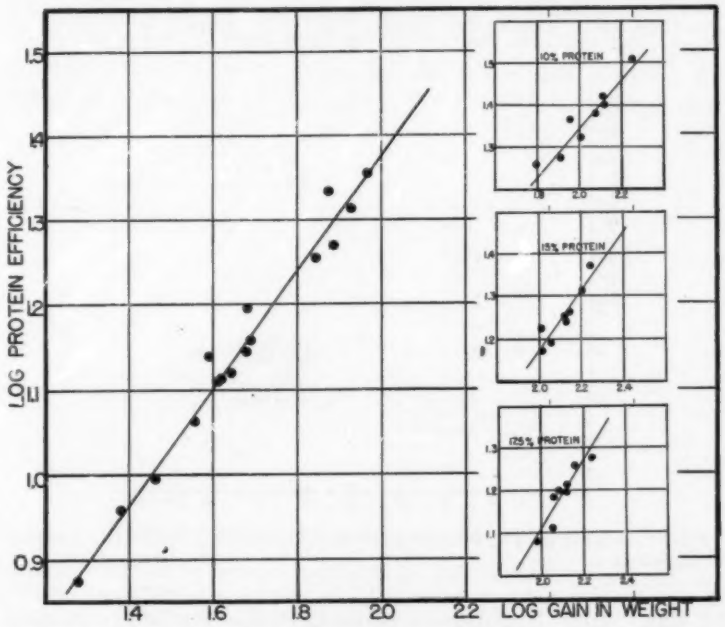


Fig. 8 The relation between protein efficiency and gain in weight in studies by Jones and Divine ('44). Insets are data from the paper by Jones and Widness ('46).

different protein mixtures were compared, all of them at a level of 9.1% protein in the diet. In the second paper (Jones and Widness, '46) 8 proteins were compared at 3 levels in the diet, namely, 10%, 15% and 17.5%. In all of these studies the correlation is very close and the points fall within the range of the standard errors of the estimated protein efficiency.

Additional examples are afforded by the recent paper of Russell et al. ('46) in which many legumes were compared as well as the effect of added methionine at 2 different levels. All of the data from this paper are shown in figure 9, and it is clear that there is a straight line relation between protein efficiency and gain in weight as in the previous data. Data obtained with chicks by Grau ('46) are shown in figure 10.

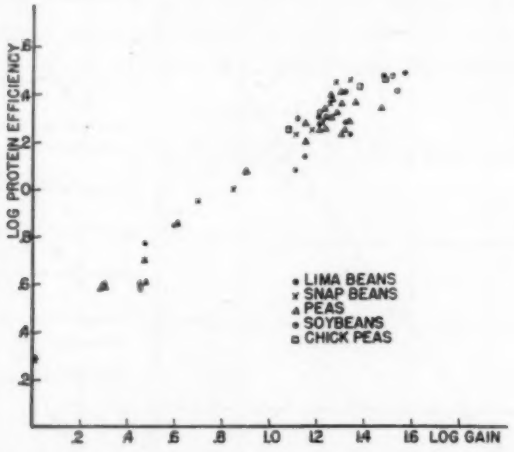


Fig. 9 The relation between protein efficiency and gain in weight in the studies by Russell et al. ('46).

In this paper 3 different proteins were studied including gluten, cottonseed and peanut proteins. Various combinations of amino acids were added to demonstrate the limiting deficiencies. A plot of these data, using food efficiency rather than protein efficiency, shows clearly that the relation is the same in chicks as in rats.

Much other data could have been selected which support the data presented in this paper. These papers were used to show that close correlation between protein efficiency and gain in weight as found under diverse conditions including many proteins, experiments involving different lengths of time, in

several laboratories, different strains of rats and even different species.

It must be stressed that this does not imply that the protein efficiency will always be the same figure for a given gain in weight. As was noted previously, the protein efficiency in figure 2 is higher relative to weight gain than in figure 1. This does not seem strange since it is well known that in repeating experiments with similar diets the absolute gain in weight may show considerable difference and protein efficiency is a function of this gain. We believe, therefore, that

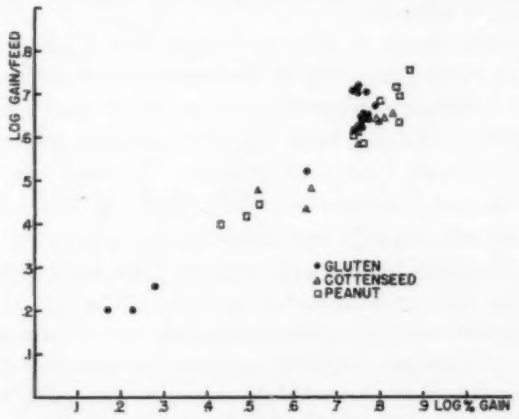


Fig. 10 The relation between food efficiency and weight gains in studies on chicks by Grau ('46).

like growth rates, protein efficiency is only relative and in the strict sense should only be used to compare the groups of animals studied at one time.

The question may be asked as to which of the 3 variables distinguishes most effectively between proteins, i.e., is 1 measure more accurate than another. In order to approach an answer to this question, variance analyses have been made of the logarithms of the 3 variables. Logarithms were used since, as previously shown, the regression lines tend to be straighter. Table 2 gives the 3 variance analyses. The 0.01

point for an F value based on 9 and 38 degrees of freedom for the greater and smaller mean square, respectively, is approximately 2.9. Consequently the variance analyses show that any 1 of the 3 variables, gain in weight, protein efficiency or amount of protein eaten, serves to bring out significant differences between the 10 proteins. The F values for gain in weight and protein efficiency are both extremely large and approximately the same size, 24.8 and 28.6. This indicates that either variable distinguishes very effectively between the proteins and it is impossible on the basis of this test to decide which is most effective.

In order to decide if gain in weight and protein efficiency can be used interchangeably in distinguishing between the proteins, the covariance between gain in weight and protein efficiency was analyzed (table 3). Comparison of the errors of estimate shows that the variances between the adjusted mean values and the observed mean values based on 9 degrees of freedom are slightly smaller than the errors of estimate determined within the protein groups. This indicates that the 2 variables, gain in weight and protein efficiency, are in fact measuring the same characteristic and that if one wishes to determine differences between proteins in terms of this characteristic, whatever it may be, one may use gain in weight alone. This is further indicated by the very high correlation

TABLE 2

Variance analyses. With respect to the 3 variables, gain in weight, protein efficiency and amount eaten.

SOURCE OF VARIATION	DEGREES OF FREEDOM	GAIN IN WEIGHT			PROTEIN EFFICIENCY			AMOUNT EATEN		
		Sum of squares	Mean squares	F	Sum of squares	Mean squares	F	Sum of squares	Mean squares	F
Total	47	6.5412			4.2360			.4119		
Between proteins	9	5.5903	.6211	24.8	3.6915	.4102	28.6	.2105	.02339	4.35
Within proteins	38	.9509	.0250		.5445	.0143		.2044	.00538	

coefficient, + 0.984, between gain in weight and protein efficiency in this experiment.

If the same sort of covariance analysis is applied to gain in weight and amount eaten the results in table 4 are obtained. The table shows that after correction for the amount eaten there is still a highly significant variance in gain in weight on the different proteins. This result is to be expected and simply means that there is a difference in the growth pro-

TABLE 3

Analysis of covariance and test of significance of adjusted protein means.[1]

SOURCE OF VARIATION	D.F.[2]	Sx^2	$S\,xy$	Sy^2	D.F.	ERRORS OF ESTIMATE			
						y from x		x from y	
						S sq.[3]	Mean square	S sq.[4]	Mean square
Total	47	6.5412	5.1817	4.2360	46	.1312		.2027	
Between proteins	9	5.5903	4.5358	3.6915					
Within proteins	38	.9509	.6459	.5445	37	.1058	.00286	.1847	.00499
Test of significance of adjusted means					9	.0254	.00282	.0180	.00200

[1] x = log gain, y = log protein efficiency. [2] Degrees of freedom.

[3] $Sy^2 - \frac{(Sxy)^2}{Sx^2}$ [4] $Sx^2 - \frac{(Sxy)^2}{Sy^2}$

TABLE 4

Analysis of covariance and test of significance of adjusted protein means.

SOURCE OF VARIATION	D.F	Sz^2	Sxz	Sx^2	D.F	ERRORS OF ESTIMATE		
						Squares[1]	Mean squares	F
Total	47	.4149	1.3608	6.4312	46	2.0781		
Between proteins	9	.2105	1.0553	5.5903				
Within proteins	38	.2044	.3055	.9509	37	.4943	.01336	
Test of significance of adjusted means					9	1.5838	.1760	13.2

[1] $x^2 = (Sxz)^2/z^2$.

moting power of proteins even though food intakes are equalized.[5]

As stated previously, the originators of this method believed that the point of maximum efficiency for each protein should be determined, and the amount of protein in the diet at these points be used in evaluating the proteins. Whether this method actually will be helpful still remains to be proven. In the final analysis the method is still one of relative classification and it is apparent that *a priori* reasoning is not sufficient grounds for stating the best method of classifying proteins. It is known already that the protein content of the diet for maximum efficiency is higher for proteins of lesser value. Thus it is not obvious that the use of the original procedure will give additional knowledge of practical value in classifying proteins over that obtained in simple feeding experiments at 1 level of protein, although this remains to be studied.

It appears to us that 1 disadvantage of the calculation of protein efficiency is that it supplies a figure for each protein which workers in the field tend to accept as characteristic of the protein. On the other hand, figures for gain in weight are always readily recognized and used in a comparative sense. Actually protein efficiency should be used in the same way and with the same reservations.

[5] The table also supplies various estimates of the correlation coefficient between gain and amount eaten. These are + 0.826 from the total, + 0.973 from the mean values, and + 0.693 from the departures within the groups. The last correlation coefficient, + 0.693, is the correlation between gain and amount eaten, independent of the type of protein fed. From this an estimate of the regression of gain in weight and amount eaten may be obtained. This regression coefficient, $\frac{\delta xz}{\delta x^2}$, is 1.4946 and not 1 as is assumed when the log protein efficiency, log x — log z, is computed. If this regression coefficient is a constant with various proteins and one desires to take account of the amount eaten, it should be done from this regression coefficient rather than by the simple computation of protein efficiency. Our data within groups are limited to decide this point but suggest that this regression coefficient is not a constant for the different proteins.

CONCLUSION

The nutritive value of 23 different proteins or protein mixtures was investigated by feeding experiments with 111 young rats. All diets contained 12% protein. An analysis of the data shows:

1. There is a very high correlation between gain in weight and protein efficiency (gain per gm of protein eaten).
2. Protein efficiency is a function of gain in weight rather than a characteristic of the protein fed.
3. Proteins are classified relative to each other in the same manner, and with equal accuracy, by either gain in weight alone or protein efficiency.
4. In studies on the relative nutritive value of various proteins using growing rats fed ad libitum, little additional information is gained by taking into account the amount of protein eaten, i.e., the calculation of protein efficiency.
5. The results are clearly supported by numerous data in the literature.

ACKNOWLEDGMENT

The technical assistance of Miss Alice Hay and Miss Virginia Kent in this study is gratefully acknowledged.

LITERATURE CITED

Boas-Fixen, M. A., J. C. D. Hutchinson and H. M. Jackson 1934 The biological value of proteins. V. The comparative biological values of the proteins of whole wheat, whole maize and maize gluten, measured by the growth of young rats. Biochem. J., *28:* 592.

Grau, C. R. 1946 Protein concentrates as amino acid sources for the chick: corn gluten meal, cottonseed meal, and peanut meal. J. Nutrition, *32:* 303.

Hegsted, D. M., J. M. McKibbin and F. J. Stare 1944 The effect of atabrine on choline deficiency in the young rat. J. Nutrition, *27:* 149.

Hegsted, D. M., R. C. Mills, C. A. Elvehjem and E. B. Hart 1941 Choline in the nutrition of chicks. J. Biol. Chem., *138:* 459.

Jones, D. B., and J. P. Divine 1944 The protein nutritional value of soybeans, peanut, and cottonseed flours and their value as supplements to wheat flour. J. Nutrition, *28:* 41.

Jones, D. B., and K. D. Widness 1946 The comparative growth-promoting value of the proteins of wheat germ, corn germ and of some other protein foods of plant and animal origin. J. Nutrition, *31:* 675.

MITCELL, H. H. 1924 The nutritive value of proteins. Physiol. Rev., *4:* 424.

———— 1942 Biological methods of measuring the protein values of foods. J. Animal Sci., *2:* 263.

OSBORNE, T. B., L. B. MENDEL AND E. L. FERRY 1919 A method of expressing numerically the growth-promoting value of proteins. J. Biol. Chem., *37:* 223.

PEARSON, K. 1897 Mathematical contributions to the theory of evolution: on a form of spurious correlation which may arise when indices are used in the measurement of organs. Proc. Royal Soc. of London, *60A:* 489.

RUSSELL, W. C., M. W. TAYLOR, T. G. MEHRHOF AND R. R. HIRSCH 1946 The nutritive value of the protein of varieties of legumes and the effect of methionine supplementation. J. Nutrition, *32:* 313.

SNEDECOR, G. W. 1946 Statistical Methods. The Iowa State College Press, Ames, Iowa.

REPRODUCTION AND LACTATION IN HIGHLY INBRED STRAINS OF MICE ON SYNTHETIC DIETS [1]

PAUL F. FENTON [2] AND GEORGE R. COWGILL,
WITH THE TECHNICAL ASSISTANCE OF MARIE A. STONE
Yale Nutrition Laboratory, Department of Physiological Chemistry, Yale University, New Haven, Connecticut

(Received for publication February 19, 1947)

The mouse has proved to be unusually valuable for the study of numerous problems in medicine and allied fields, notably those related to cancer and certain infectious diseases, concerning which there have been some suggestions in the literature that dietary factors may play a role. Unfortunately, this species has not been as well standardized nor have its nutritional requirements been as well elucidated as have those of the rat. Therefore it has been difficult to study the problems of cancer and infectious disease on the mouse using highly artificial diets so constructed and controlled as to have only one variable of interest. In this institution many highly inbred strains of mice with characteristics important for students of cancer have been developed by Dr. L. C. Strong. The origin of a number of these strains has been discussed elsewhere (Strong, '42).

In some preliminary dietary studies of this general problem Rogers, McElroy and Cowgill ('42) succeeded in carrying mice of the C_{57} strain (characterized by low incidence of

[1] The expenses of this study were defrayed by a grant from Nutrition Foundation, Inc., and also by grants from the American Cancer Society and The Anna Fuller Fund.

[2] Nutrition Foundation Fellow, 1945–46.

spontaneous breast cancer) to the second generation of offspring (F_2) on a purified diet containing as the component of uncertain composition a "rice polish filtrate factor II." Efforts to repeat this with other strains, however, met with failure. Foster et al. ('43) made considerable progress in this field. Cerecedo and Vinson ('44) reported that the poor lactation of mice on purified diets could be improved by the addition of a crude folic acid preparation. This work was recently repeated using crystalline folic acid (Cerecedo and Mirone, '47). The nutritional requirements of the mouse as recorded in the literature up to 1944 have been reviewed by Morris ('44).

In view of the apparent strain differences in nutritional requirements already observed by Rogers, McElroy and Cowgill ('42), it was decided to investigate this question in greater detail. We have already demonstrated [3] that the riboflavin requirement for growth is less for mice of the C_{57} strain than for the A strain (albinos with high incidence of spontaneous mammary tumors among mated females). Others have reported that it is much more difficult to maintain the A strain presumably because of either poor reproduction or poor lactation, or both. In this paper are reported favorable results obtained with respect to reproduction and lactation in the C_{57} and A strains fed highly artificial rations.

EXPERIMENTAL

This study was begun with mice of the C_{57} and A strains received from Dr. L. C. Strong and Dr. A. Gorbman.[4] Most of the animals were 21 to 28 days old at the time. One group of each strain was fed commercial stock rations [5] (in a few instances experimental stock rations); the other group received diet 101. The composition of all synthetic rations is given in table 1. A few animals were raised from weaning on diet 104. In later experiments offspring of these groups of

[3] To be published.
[4] Department of Anatomy, Yale University.
[5] Purina Dog Chow, later Purina Laboratory Chow.

TABLE 1

Composition of diets.

DIET NO.	101	104	108	111	112	114	116	119	132	133
Casein — Labco, gm	23	23	23	23	23	30	23	23	30	30
Dextrose, C.P., gm	60	57	57	60	60	45	60	60	52.5	
Dextrin, gm										52.5
Crisco, gm	10	10	10	10	10	15	10	10		
Corn oil, gm									10	10
Salts — Sure's no. 2, gm	5	5	5	5	5	7	5	5	5	5
Ruffex, gm	2	2	2	2	2	3	2	2	2	2
Cystine, gm					0.2				0.5	0.5
Rice polish filtrate factor II,[1] gm			3							
Liver powder,[2] gm		3								
Cod liver oil concentrate, gm	0.2	0.2	0.2	0.2	0.2	0.3	0.2	0.2	0.2	0.2
Linoleic acid, gm	0.27	0.27	0.27	0.27	0.27	0.41	0.27	0.27		
Choline, mg	150	150	150	150	150	196	150	150	150	150
Paba, mg	100	100	100	100	100	131	100	100	100	100
Inositol, mg	100	100	100	100	100	131	100	100	100	100
Thiamine, mg	0.5	0.5	0.5	0.5	0.5	0.65	0.5	0.5	0.5	0.5
Pyridoxine, mg	0.5	0.5	0.5	0.5	0.5	0.65	0.5	0.5	0.5	0.5
Riboflavin, mg	1	1	1	1	1	1.3	1	1	1	1
Nicotinic acid, mg	1	1	1	1	1	1.3	1	1	1	1
Calcium pantothenate, mg	3	3	3	3	3	3.9	3	3	3	3
Biotin, mg				0.016			0.015		0.02	0.02
Folic acid, mg							0.24	0.24	0.05	0.05
α-Tocopherol, mg	6	6	6	6	6	9	6	6	6	6
2-methyl-1,4-naphthoquinone, mg									1	1

[1] The Borden Co.

[2] The Wilson Laboratories.

mice (F_1 generation) were used. Since the belief is held in some quarters that mice will mate satisfactorily only if the male and female are raised together in the same cage, this practice was followed in the earlier experiments. From a nutritional viewpoint, if growth performance is to be measured, this system has obvious disadvantages. Consequently in most of our experiments the animals were raised from weaning in individual cages and were mated after they had reached an age of at least 70 days. The cages have wire screen bottoms and fronts, are suspended from metal shelves and are of all-metal construction. Food and water were supplied ad libitum. The diets were prepared usually at least once a week, often more frequently; between feedings they were stored in the cold. Growth rates on the stock rations and on diets 101, 104 and 114 were excellent. Growth performance on the remaining diets has not been determined.

When an animal was observed to be pregnant, it was separated from the male and placed in an individual cage supplied with nesting paper. The details regarding exact equipping of these cages were evolved gradually in the course of the early experiments. A piece of wire screen the same size as the rectangular cage bottom but of somewhat smaller mesh was turned up at the front and back to form a basket (the sides of the cage serving simultaneously as the sides of the basket). Into this the nesting paper was placed. This nest occupied the rear half of the cage. The food cup was placed in the front half of the cage, and the water bottle was attached to the front of the cage with a glass delivery tube passing through the screen to the inside. The chief advantages of this system are (a) that the nesting paper is usually kept away from the water tube and (b) that the animal quickly habituates itself to dropping the feces in the front part of the cage where they can fall through the bottom. Early experiments showed that nesting paper placed indiscriminately in the cage was invariably pushed against the water bottle outlet causing a rapid emptying. The prevention of coprophagy is a fundamental problem in nutrition and has not

been satisfactorily solved even for simple growth or maintenance experiments. The problem becomes even more acute when it is necessary to prevent the newly born mice from falling out of the cage. The method described here has, by observation, reduced coprophagy to a minimum. It is recognized, however, that the opportunities for ingestion of feces are greater than they would be in the original wire-bottom cage.

The cages containing pregnant animals were examined at least once a day. When the young were born, the female was weighed as was the entire litter. The number of offspring was reduced to 8 at birth and to 6 at the end of 1 week. The lactating females and the litters were weighed once a week and the young weaned at 21 days of age. After weaning or after the death of the last offspring in cases of lactation failure, the female was allowed to rest at least 2 weeks before being remated. Biotin assays were done by the microbiological method of Wright and Skeggs ('44) using *L. arabinosus*. This procedure was checked with an *L. casei* method. Numerous recovery experiments were carried out to test the validity of the assays, and the recoveries were entirely satisfactory. Hydrolysis of the tissue with 6 N acid was found to cause some loss of biotin, and therefore hydrolysis was done with N acid which was found to give maximal yields of biotin.

RESULTS

The data on reproduction and lactation are summarized in tables 2, 3 and 4. Mice raised on stock diets have given satisfactory breeding performance during the year. On the other hand on the purified diet (no. 101) 21 animals produced litters, 20 of which died within 3 days after birth. One litter (C_{57}) survived 14 days (table 2). Four females had been set aside as pregnant but no litters were found. It seems likely that the offspring were consumed by the mother prior to the daily inspection. No pregnancy was observed in 5 of the animals on the purified diet. Three litters were accidentally lost. When the dead young were analyzed for biotin, they

contained on the average 0.064 μg per gm of wet tissue (49 determinations on 14 animals; range 0.032 to 0.094 μg). Offspring of animals on stock rations contained on the average 0.172 μg of biotin per gm (53 determinations on 6 animals; range 0.159 to 0.184 μg). Earlier work carried out in this laboratory by A. E. H. Houk [6] and G. Keefe had shown essentially the same results. Mice of the C_{57} strain were fed rations nearly identical with our diet 101. A total of 52 litters were obtained but only 3 young were successfully weaned.

Following a 2-week rest period the females were remated. Some were continued on diet 101; others which had been raised on diet 101 were put on diets 104, 108, 111, 112, 114, 116 and 119. For subsequent matings these animals were further interchanged among the several diets. On diet 104 (liver supplement) 3 litters were weaned and the survival time of several others was increased. The addition of 16 μg of biotin (biotin concentrate) per 100 gm of diet had no beneficial effect under the conditions of this experiment. On diet 112 (cystine supplement) 2 litters, a total of 3 young were weaned (both by the same animal). Increasing the protein, fat, vitamin, salt and roughage content of the diet at the expense of carbohydrate (diet 114) was without effect. Addition of folic acid (diet 119) made it possible for 1 litter to be weaned. When both biotin and folic acid were added (diet 116), 4 litters were weaned by 2 females. Two animals were changed from the synthetic diet to a stock ration; both successfully weaned litters after this change. One of these animals was later returned to diet 101 after which the next litter died soon after birth. Young born to the animals which had been continued on diet 101 all died within 3 days after birth. Three animals raised from weaning on diet 104 were mated, and 1 litter of 5 young were successfully weaned. Five animals of our F_1 generation (from stock colony) were raised from weaning on diet 101 and placed on diet 116 at the time of mating. Of 5 litters born 1 was successfully weaned. Five other animals were raised from weaning on diet 116, but none succeeded in weaning their first

[6] Nutrition Foundation Fellow, 1943–44.

TABLE 2

Lactation performance of mice raised on synthetic diets.

DIET	FIRST GENERATION						F_1 GENERATION					
	First litter			Subsequent litters			First litter			Subsequent litters		
	Litters		Young weaned	Litters		Young weaned	Litters		Young weaned	Litters		Young weaned
	born	weaned		born	weaned		born	weaned		born	weaned	
101	21	0	0	4	0	0						
104				6	3	11						
108				4	1	6						
111				8	0	0						
112				6	2	3						
114				4	0	0						
116				10	4	18	10	1	2			
119				4	1	3						
L[1]				2	2	8						
132							10	4	19	8	2	9
133							6	1	6	19	12	60

[1]Purina Laboratory Chow.

TABLE 3

Lactation performance of mice raised on stock rations and placed on diet 101 at the second mating.

ANIMAL NO.	STRAIN	FIRST LITTER DIET L[1]			SECOND LITTER DIET 101			THIRD LITTER			
		No. weaned	Total wt.	No. dead	No. weaned	Total wt.	No. dead	Diet	No. weaned	Total wt.	No. dead
			gm			gm				gm	
1233	C_{57}	4	28.3	7	(young dead in 16 days)		11				
1240	C_{57}	2	14.5	5	(young lost accidentally)			101	(young dead in 2 days)		8
1249	C_{57}	2	14.6	2	6	30.3	1	101	(young dead in 3 days)		2
1250	C_{57}	6	49.2	0	6	41.8	0	101	1	4.6	1
1253	C_{57}	6	46.4	1	6	39.3	0	101	6	38.2	1
1232	A	5	30.3	0	(young dead in 5 days)		3	L[1]	5	44.3	0
1239	A	6	41.1	0	5	27.9	2	L	6	48.8	0
1240	A	6	39.4	0	(young dead in 1 day)		6	L	5	52.7	0

[1]Purina Laboratory Chow.

TABLE 4

Weight changes of females and offspring on diets 132 and 133.

	DIET 132: 6 LITTERS				DIET 133: 13 LITTERS			
Weeks after parturition	0	1	2	3	0	1	2	3
Average wt. of females, gm	26.3	27.7	29.3	27.3	27.0	27.3	26.9	26.4
Total no. of young	36	32	30	30	82	76	70	66
Average litter wt., gm	7.9	17.8	33.0	44.5	8.3	20.8	34.8	42.5
Average individual wt. of young, gm	1.3	3.3	6.6	8.8	1.3	3.6	6.5	8.4

litters. When these animals were later placed on diets 132 and 133, lactation performance was far better.

In 2 instances the young born to a female on diet 101 were exchanged with the young of a female on stock ration. Both times the stock animals successfully weaned the young, while 1 female on the synthetic diet carried 1 offspring to age 21 days when it weighed only 3.7 gm.

Three stock animals were transferred to diet 101 at parturition. All 3 litters were successfully weaned. Eight females which had been raised on stock rations and had successfully weaned 1 litter each were placed on diet 101 when they were mated a second time. The definitely adverse effects of this change on most of the animals are shown in table 3 along with results of subsequent matings.

The females of an F_1 generation group raised on synthetic diets were mated when at least 75 days old and were then placed on diets 132 and 133. The results are shown in table 2 in which are also included those F_1 animals which had been bred once when on diet 116. By employing diets 116, 132 and 133 it has been possible to carry a number of animals to the third generation entirely on synthetic diets. In table 4 are shown the weight changes of females and litters successfully raised on diets 132 and 133.

DISCUSSION

It has been shown that diet 101 (which supports excellent growth) is utterly inadequate to permit successful weaning of the offspring. The fact that offspring of females on this diet could be successfully raised if foster-nursed by females on stock diets suggests that the problem is chiefly one of lactation. The complete failure of our efforts to secure lactation on diets 101 and 114 is most interesting in view of the partial success reported by Cerecedo and Vinson ('44) with their diets R-5 and C-28, which closely resemble ours in composition. The difference in results is perhaps partly due to differences in housing the breeding colony. The partial success obtained by Foster et al. ('43) may perhaps be ascribed to

their use of vitamin K in their diet since Jukes ('40) has stressed the importance of this factor during the lactation period of the rat. Kennedy and Palmer ('45) showed biotin to be essential for reproduction and lactation of the rat.

The addition of a liver powder (diet 104) resulted in some improvement in lactation, but performance was not equal to that obtained on stock rations. The incorporation of cystine alone (diet 112) was of extremely doubtful value. Biotin concentrate was ineffective at the level employed (diet 111). Merely increasing some of the constituents of diet 101 at the expense of carbohydrate (diet 114) was equally futile. Only when folic acid or folic acid and biotin were added to diet 101 was any appreciable success obtained.

The fact that females which had been on stock rations could be transferred to diet 101 at parturition and could successfully wean their litters shows that the essential factor or factors were depleted at a rather slow rate. This was again brought out in the experiments in which stock animals were placed on diet 101 at the time of second mating. In these experiments the C_{37} strain appeared to do better than the A strain.

After the partial success of diets 116 and 119 it was decided to prepare rations which contained in addition to the ingredients of diet 101 most of the factors which have been reported to be important in lactation. The feeding of these new diets (132 and 133) did indeed result in markedly improved reproduction and lactation performance. It remains to be determined which of the dietary components may be safely omitted. Further modification of the diet can be expected to bring about further improvement in reproduction and lactation.

SUMMARY AND CONCLUSIONS

Reproduction and lactation has been studied in highly inbred strains of mice fed purified diets. A diet containing vitamins A, D and E as well as thiamine, riboflavin, nicotinic acid, pantothenic acid, pyridoxine, choline, inositol and p-aminobenzoic acid was utterly inadequate for this purpose. The problem appears to be one of lactation. Addition of folic acid and folic

acid plus biotin gave some success. Addition of biotin, folic acid, cystine and vitamin K gave much better results.

ACKNOWLEDGMENTS

We wish to thank Dr. D. F. Robertson of Merck and Co. for furnishing us with B-complex vitamins, particularly biotin, and Dr. T. H. Jukes of Lederle Laboratories who provided us with folic acid.

LITERATURE CITED

CERECEDO, L. R., AND L. MIRONE 1947 The beneficial effect of folic acid (Lactobacillus casei Factor) on lactation in mice maintained on highly purified diets. Arch. Biochem., *12:* 154.

CERECEDO, L. R., AND L. J. VINSON 1944 Growth, reproduction, and lactation in mice on highly purified diets, and the effect of folic acid concentrates on lactation Arch. Biochem., *5:* 157.

FOSTER, C., J. H. JONES, F. DORFMAN AND R. S. KOBLER 1943 Reproduction and lactation of mice on highly purified diets. J. Nutrition, *25:* 161.

JUKES, T. H. 1940 Reproduction in rats on synthetic B-complex supplement. Proc. Soc. Exp. Biol. Med., *45:* 625.

KENNEDY, C., AND L. S. PALMER 1945 Biotin deficiency in relation to reproduction and lactation. Arch. Biochem., *7:* 9.

MORRIS, H. P. 1944 Review of the nutritive requirements of normal mice for growth, maintenance, reproduction, and lactation. J. Nat. Cancer Inst., *5:* 115.

ROGERS, L. K., L. W. MCELROY AND G. R. COWGILL 1942 Successful rearing of a second generation of mice on an artificial diet. Science, *95:* 203.

STRONG, L. C. 1942 The origin of some inbred mice. Cancer Research, *2:* 531.

WRIGHT, L. D., AND H. R. SKEGGS 1944 Determination of biotin with Lactobacillus arabinosus. Proc. Soc. Exp. Biol. Med., *56:* 95.

INDEX

ABSORPTION and blood levels of vitamin A in children with keratosis follicularis ... 633
—— —— storage of vitamin A in the liver of the rat ... 53
—— of radioactive iron by children 7–10 years of age ... 107
—— —— vitamin A and carotene by patients with sprue, influence of pteroylglutamic acid (a member of vitamin M group) on ... 243
—— rate of, and coefficient of digestibility of various natural and artificial fats, comparison of, as influenced by melting point ... 187
Adolescent children, ascorbic acid metabolism of, study of ... 529
Adult albino rats, protein-depleted, mixtures of purified amino acids in, dietary utilization of. Studies in amino acid utilization. I. ... 65
—— dog, utilization of nitrogen in, some effects of methionine on ... 361
—— rats, young, maintained on restricted calories, weight loss, mortality and recovery in. Effect of fat level of diet on general nutrition. III. ... 641
Adults, normal, on three levels of intake, ascorbic acid in, tissue reserves of ... 479
ALBANESE, ANTHONY A. See Cox, Mueller, Elman, Albanese, Kemerer, Barton and Holt ... 437
Albino mice, use of, to determine utilization of calcium of dehydrated carrots and cabbage ... 195
—— rat, biotin balance in ... 517
—— rats, protein-depleted adult, mixtures of purified amino acids in, dietary utilization of. Studies in amino acid utilization. I. ... 65
ALLISON, JAMES B., JOHN A. ANDERSON AND ROBERT D. SEELEY. Some effects of methionine on the utilization of nitrogen in the adult dog ... 361
Amino acid content of feeds. I. Leucine, valine, isoleucine and phenylalanine ... 553
—— —— utilization, studies in. I. Dietary utilization of mixtures of purified amino acids in protein-depleted adult albino rats ... 65
—— —— —— —— —— —— II. Essential amino acids as a source of plasma protein and erythrocytes in the hypoproteinemic rat ... 85
—— —— in nitrogen metabolism with particular reference to the role of methionine ... 389
—— —— —— the urine of human subjects fed eggs or soy beans ... 209
—— —— purified, mixtures of, dietary utilization of, in protein-depleted adult albino rats. Studies in amino acid utilization. I. ... 65
—— —— required to supplement linseed protein for chick growth ... 313
ANDERSON, JOHN A. See Allison, Anderson and Seeley ... 361
Animal protein, diet free of, response of chickens to, inborn characteristic determining ... 319
Artificial and natural fats, various, coefficient of digestibility and rate of absorption of, comparison of, as influenced by melting point ... 187
Ascorbic acid metabolism, study of, of adolescent children ... 529
—— —— plasma levels and urinary excretion of, in women during menstrual cycle ... 511
—— —— renal threshold for. A modified method for its estimation with results for twelve adult subjects ... 505
—— —— tissue reserves of, in normal adults on three levels of intake ... 479
ASENJO, CONRADO F. See Goyco and Asenjo ... 593
AUGUR, MARIAN V., HILDA S. ROLLMAN AND HARRY J. DEUEL, JR. The effect of crude lecithin on the coefficient of digestibility and rate of absorption of fat ... 177
Autoclaving soybean oil meal, influence of, on digestibility of proteins ... 661

BAKER, ELIZABETH F. See Kratzer, Williams and Baker ... 313
BARTON, RAYMOND W. See Cox, Mueller, Elman, Albanese, Kemerer, Barton and Holt ... 437
BAUMANN, CARL A. See Steele, Sauberlich, Reynolds and Baumann ... 209
B-complex vitamins, other, effect of supplementing enriched flour with. Improving the nutritive value of flour. I. ... 301
B_6-deficiency, role of, in the tryptophane-niacin relationships in rats ... 561
Beans, soy, or eggs, human subjects fed, urine of, amino acids in ... 209
BEESON, WILLIAM M. See Lehrer, Woods and Beeson ... 469
BELT, MARGARET. See Jukes, Stokstad and Belt ... 1
BENDANA-BROWN, ADELAIDA M. AND BARKER H. BROWN. The use of albino mice to determine the utilization of the calcium of dehydrated carrots and cabbage ... 195
BENDITT, EARL P., ELEANOR M. HUMPHREYS, ROBERT L. STRAUBE, ROBERT W. WISSLER AND CLYDE H. STEFFEE. Studies in amino acid utilization. II. Essential amino acids as a source of plasma protein and erythrocytes in the hypoproteinemic rat ... 85
BERG, JOSEPH L., EDGAR R. PUND, VIRGIL P. SYDENSTRICKER, WALTER K. HALL, LESTER L. BOWLES AND CHARLES W. HOCK. The formation of capillaries and other tissue changes in the cornea of the methionine-deficient rat ... 271

Bioassay of vitamin B_6 in natural materials 121
Biochemical defect underlying the nutritional failure of young rats on diets containing excessive quantities of lactose or galactose 221
Biotin balance in the albino rat 517
BIRD, HERBERT R., MAX RUBIN AND ALBERT C. GROSCHKE. An inborn characteristic determining the response of chickens to a diet free of animal protein 319
—— ORSON D. See Lemley, Brown, Bird and Emmett 53
Blood constituents, certain, work performance, and respiratory exchange after isocaloric meals of low and high carbohydrate content 287
—— diets, breast milk and urine, vitamin C in, of nursing mothers. Metabolism of women during reproductive cycle. XI. 601
—— levels and absorption of vitamin A in children with keratosis follicularis 633
BOER, J., B. C. P. JANSEN AND A. KENTIE. On the growth-promoting factor for rats present in summer butter 339
H. W. KNOL. The growth-promoting action of vaccenic acid 359
BOWLES, LESTER L. See Berg, Pund, Sydenstricker, Hall, Bowles and Hock 271
Breast milk, diets, blood and urine, vitamin C in, of nursing mothers. Metabolism of women during reproductive cycle. XI. 601
BRODY, SAMUEL. See Kibler, Brody and Worstell 331
BROWN, BARKER H. See Bendafia-Brown and Brown 195
—— RAYMOND A. See Lemley, Brown, Bird and Emmett 53
BRUSH, MIRIAM K., WANDA J. WILLMAN AND PEARL P. SWANSON. Amino acids in nitrogen metabolims with particular reference to the role of methionine 389
BURR, G. O. See Loeb and Burr 541
Butter, summer, rats present in, on growth-promoting factor for 339
Butters, retail, vitamin A and carotene content of, seasonal variations in 39

CALCIUM, utilization of, use of albino mice to determine, of dehydrated carrots and cabbage 195
Calf, dairy, riboflavin deficiency in 263
Calories, restricted, weanling rats maintained on, growth, mortality and recovery in. Effect of fat level of the diet on general nutrition. II. 583
—— —— young adult rats maintained on, weight loss, mortality and recovery in. Effect of fat level of diet on general nutrition. III. 641
CANNON, PAUL R. See Frazier, Wissler, Steffee, Woolridge and Cannon 65
Capillaries and other tissue changes, formation of, in cornea of methionine-deficient rat 271
Carbohydrate content, low and high, isocaloric meals of, work performance, respiratory exchange, and certain blood constituents after 287
Carotene and vitamin A, absorption of, by patients with sprue, influence of pteroylglutamic acid (a member of the vitamin M group) on 243
—— content and vitamin A, seasonal variations in, of retail butters 39
—— vitamin A, and fat, output of, effect of tocopherol supplementation on, by dairy cows. Covitamin studies. VI. 411
Carrots and cabbage, dehydrated, utilization of calcium of use of albino mice to determine 195
Casein hydrolysate, enzymic, effect of adding methionine to; nitrogen retention studies on rats, dogs and man 437
Cereal phosphorus, utilization of, and source of vitamin D, relationship between, by the poult. Phosphorus in poultry nutrition. III. 13
Chick growth, linseed protein for, amino acids required to supplement 313
Chickens, response of, inborn characteristic determining, to a diet free of animal protein 319
Children, adolescent, ascorbic acid metabolism of, study of 529
—— 7–10 years of age, absorption of radioactive iron by 107
—— with keratosis follicularis, blood levels and absorption of vitamin A in 633
Choline, protein, fat, methionine, cystine, nicotinic acid and tryptophane, some physiological relationships of 155
CODIE, JOHN F. See Scheer, Codie and Deuel 641
Coefficient of digestibility and rate of absorption of fat, crude lecithin on, effect of 177
Comparison of the coefficient of digestibility and the rate of absorption of various natural and artificial fats as influenced by melting point 187
Composition of rats, study of sex differences in, with emphasis on lipid component. Sex difference in susceptibility to essential fatty acid deficiency with high and low fat diets 541
Conjoint effects of varied intakes of thiamine and vitamin A on rats 459
COOK, MARY B. See Darby, Hahn, Kaser, Steinkamp, Densen and Cook 107
Cooking and curing, effect of, of lysine content of pork luncheon meat 235
Copper and manganese storage in the rat, rabbit and guinea pig 143
Coprophagy, growth studies with rats kept under conditions which prevent 129
Corn, relation of, to requirement of rat for dietary nicotinic acid 169
Cornea, formation of capillaries and other tissue changes in, of methionine-deficient rat 271
Cottonseed oil or margarine fat, diets containing various levels of, rats receiving ad libitum, growth, reproduction and physical capacity of. Effect of fat level of the diet on general nutrition. I. 569
Covitamin studies. VI. Effect of tocopherol supplementation on the output of vitamin A, carotene, and fat by dairy cows 411
COWGILL, GEORGE R. See Fenton and Cowgill 703

COX, WARREN M., JR., ARTHUR J. MUELLER, ROBERT ELMAN, ANTHONY A. ALBANESE, KENNETH S. KEMMERER, RAYMOND W. BARTON AND LUTHER E. HOLT, JR. Nitrogen retention studies on rats, dogs and man; the effect of adding methionine to an enzymic casein hydrolysate 437
CRAMPTON, EARL W. The growth of the odontoblasts of the incisor tooth as a criterion of the vitamin C intake of the guinea pig 491
CROCKETT, MARY E., AND HARRY J. DEUEL, JR. A comparison of the coefficient of digestibility and the rate of absorption of various natural and artificial fats as influenced by melting point 187
Curing and cooking, effect of, of lysine content of pork luncheon meat 235
Cycle, menstrual, ascorbic acid in women during, plasma levels and urinary excretion of 511
—— reproductive, metabolism of women during. XII. Effect of multivitamin supplements on the vitamin C utilization of nursing mothers 621
—— —— —— —— —— —— XI. Vitamin C in diets, breast milk, blood and urine of nursing mothers 601
Cystine, protein, fat, choline, methionine, nicotinic acid and tryptophane, some physiological relationships of 155

DAIRY calf, riboflavin deficiency in 263
—— cows, output of vitamin A, carotene, and fat by, effect of tocopherol supplementation on. Covitamin studies. VI. 411
DANIEL, FREDERICK K., AND ELLERY H. HARVEY. Some observations on the nutritional value of dialyzed whey solids 429
DARBY, WILLIAM J., MARGARET M. KASER AND EDGAR JONES. The influence of pteroylglutamic acid (a member of the vitamin M group) on the absorption of vitamin A and carotene by patients with sprue . 243
—— —— —— PAUL F. HAHN, MARGARET M. KASER, RUTH C. STEINKAMP, PAUL M. DENSEN AND MARY B. COOK. The absorption of radioactive iron by children 7–10 years of age 107
DAVEY, BESSIE L. See Storvick, Fincke, Quinn and Davey ... 529
DAY, HARRY G., AND BLANCHE E. SKIDMORE. Some effects of dietary zinc deficiency in the mouse 27
Defect, biochemical, underlying nutritional failure of young rats on diets containing excessive quantities of lactose or galactose ... 221
Deficiencies of certain vitamins as studied with turkey poults on a purified diet. I. Pteroylglutamic acid, riboflavin, niacin and inositol 1
Deficiency, dietary zinc, some effects of, in the mouse 27
—— essential fatty acid, sex difference in susceptibility to, with high and low fat diets. Study of sex differences in the composition of rats, with emphasis on the lipid component 541
—— riboflavin, in dairy calf 263
DELANEY, MARGARET A. See Haines, Klosterman, Hauck, Delaney and Kline 479
DENSEN, PAUL M. See Darby, Hahn, Kaser, Steinkamp, Densen and Cook 107
DERSE, PHILIP H. See Geyer, Geyer, Derse, Zinkin, Elvehjem and Hart 129
DEUEL, HARRY J., JR., EMILY R. MESERVE, EVELYN E. STRAUB, CORNELIA HENDRICK AND BRADLEY T. SCHEER. The effect of fat level of the diet on general nutrition. I. Growth, reproduction and physical capacity of rats receiving diets containing various levels of cottonseed oil or margarine fat ad libitum 569
—— —— —— —— —— See Augur, Rollman and Deuel 177
—— —— —— —— —— Crockett and Deuel 187
—— —— —— —— —— Johnson, Deuel, Morehouse and Mehl ... 371
—— —— —— —— —— Scheer, Codie and Deuel 641
—— —— —— —— —— Soule, Fields and Deuel 583
Dialyzed whey solids, nutritional value of, some observations on 429
Diet(s), breast milk, blood and urine, vitamin C in, of nursing mothers. Metabolism of women during reproductive cycle. XI. 601
—— containing excessive quantities of lactose or galactose, young rats on, nutritional failure of, biochemical defect underlying 221
—— —— various levels of cottonseed oil or margarine fat, rats receiving ad libitum, growth, reproduction and physical capacity of. Effect of fat level of the diet on general nutrition. I. 569
—— fat, high and low, sex difference in susceptibility to essential fatty acid deficiency with. Study of sex differences in the composition of rats, with emphasis on the lipid component 541
—— —— level of, effect of, on general nutrition. II. Growth, mortality and recovery in weanling rats maintained on restricted calories 583
—— —— —— —— —— —— —— —— I. Growth, reproduction and physical capacity of rats receiving diets containing various levels of cottonseed oil or margarine fat ad libitum 569
—— —— —— —— —— —— —— —— III. Weight loss, mortality and recovery in young rats maintained on restricted calories 641
—— free of animal protein, response of chickens to, inborn characteristic determining 319
—— of constant protein content, study of, relation between protein efficiency and gain in weight on 685
—— purified, turkey poults on, deficiencies of certain vitamins as studied with. I. Pteroylglutamic acid, riboflavin, niacin and inositol .. 1
—— reproduction and lactation in, highly inbred strains of mice on synthetic 703

Dietary nicotinic acid, requirement of rat for, relation of corn to 169
—— utilization of mixtures of purified amino acids in protein-depleted adult albino rats. Studies in amino acid utilization. I. 65
—— zinc deficiency, some effects of, in the mouse 27
Digestibility, coefficient of, and rate of absorption of fat, crude lecithin on, effect of 177
—— —— —— —— —— —— —— ——
—— various natural and artificial fats, comparison of, as influenced by melting point 187
—— of proteins, influence of autoclaving soybean oil meal on 661
Dog, adult, utilization of nitrogen in, some effects of methionine on 361
Dogs, rats and man, nitrogen retention studies on; effect of adding methionine to an enzymic casein hydrolysate 437
Dye, Marie, Thelma Porter and Eunice Kelly. The conjoint effects of varied intakes of thiamine and vitamin A on rats 459
Dystrophy, muscular, relative activity of tocopherols in curing, in rabbits 95

EFFECT of cooking and curing of lysine content of pork luncheon meat 235
—— —— crude lecithin on the coefficient of digestibility and rate of absorption of fat 177
—— —— fat level of the diet on general nutrition. II. Growth, mortality and recovery in weanling rats maintained on restricted calories 583
—— —— —— —— —— —— —— ——
—— —— —— I. Growth, reproduction and physical capacity of rats receiving diets containing various levels of cottonseed oil or margarine fat ad libitum 569
—— —— —— —— —— —— —— ——
—— —— —— III. Weight loss, mortality and recovery in young adult rats maintained on restricted calories 641
—— —— methionine upon the urinary nitrogen in men at normal and low levels of protein intake 371
—— —— multi-vitamin supplements on the vitamin C utilization of nursing mothers. Metabolism of women during the reproductive cycle. XII. 621
—— —— source, particle size, and method of grinding. Nutritive value of keratin. III. 673
—— —— supplementing enriched flour with other B-complex vitamins. Improving the nutritive value of flour. I. 301
—— —— tocopherol supplementation on the output of vitamins A, carotene, and fat by dairy cows. Covitamin studies. VI. 411
Efficiency, protein, and gain in weight, relation between, study of, on diets of constant protein content 685
Eggs or soy beans, human subjects fed, urine of, amino acids in 209
ELLENBERGER, HERMAN A., NOLLIE B. GUERRANT AND OLIVER B. FARDIG. Seasonal variations in the vitamin A and the carotene content of retail butters 39
ELMAN, ROBERT. See COX, Mueller, Elman, Albanese, Kemmerer, Barton and Holt 437
ELVEHJEM, CONRAD A. See Geyer, Geyer, Derse, Zinkin, Elvehjem and Hart 129
—— —— —— —— Newell and Elvehjem 673
—— —— —— —— Sarma, Snell and Elvehjem 121
EMMETT, ARTHUR D. See Lemley, Brown, Bird and Emmett 53
Enriched flour, effect of supplementing, with other B-complex vitamins. Improving the nutritive value of flour. I. 301
Enzymic casein hydrolysate, effect of adding methionine to; nitrogen retention studies on rats, dogs and man 437
Erythrocytes and plasma protein, essential amino acids as a source of, in hypoproteinemic rat. Studies in amino acid utilization. II. 85
EVANS, ROBERT J., JAMES MCGINNIS AND J. L. ST. JOHN. The influence of autoclaving soybean oil meal on the digestibility of the proteins 661
Excretion, urinary, and plasma levels of ascorbic acid in women during the menstrual cycle 511

FARDIG, OLIVER B. See Ellenberger, Guerrant and Fardig 39
Fat, choline, protein, methionine, cystine, nicotinic acid and tryptophane, some physiological relationships of 155
—— coefficient of digestibility and rate of absorption of, crude lecithin on, effect of 177
—— diets, high and low, sex difference in susceptibility to essential fatty acid deficiency with. Study of sex differences in the composition of rats, with emphasis on the lipid component 541
—— level of diet, effect of, on general nutrition. II. Growth, mortality and recovery in weanling rats maintained on restricted calories 583
—— —— —— —— —— —— —— ——
—— —— I. Growth, reproduction and physical capacity of rats receiving diets containing various levels of cottonseed oil or margarine fat ad libitum 569
—— —— —— —— —— —— —— ——
—— —— III. Weight loss, mortality and recovery in young adult rats maintained on restricted calories 641
—— margarine, or cottonseed oil, diets containing various levels of, rats receiving ad libitum, growth, reproduction and physical capacity of. Effect of fat level of the diet on general nutrition. I. 569
—— vitamin A, and carotene, output of, effect of tocopherol supplementation on, by dairy cows. Covitamin studies. VI. 411
Fats, various natural and artificial, coefficient of digestibility and rate of absorption of, comparison of, as influenced by melting point 187

Fatty acid deficiency, essential, sex difference in susceptibility to, with high and low fat diets. Study of sex differences in the composition of rats, with emphasis on the lipid component ... 541
Feeds, amino acid content of. I. Leucine, valine, isoleucine and phenylalanine ... 553
FENTON, PAUL F., AND GEORGE R. COWGILL, with the technical assistance of Marie A. Stone. Reproduction and lactation in highly inbred strains of mice on synthetic diets ... 703
FIELDS, MAX. See Scheer, Soule, Fields and Deuel ... 583
FINCKE, MARGARET L. See Storvick, Fincke, Quinn and Deuel ... 529
Flour, enriched, effect of supplementing, with other B-complex vitamins. Improving the nutritive value of flour. I. ... 301
Follicularis, keratosis, children with, blood levels and absorption of vitamin A in ... 633
Food yeast, net protein value of ... 593
Formation of capillaries and other tissue changes in the cornea of the methionine-deficient rat ... 271
FRAZIER, LAURENCE E., ROBERT W. WISSLER, CLYDE H. STEFFEE, ROBERT L. WOOLRIDGE AND PAUL R. CANNON. Studies in amino acid utilization. I. The dietary utilization of mixtures of purified amino acids in protein-depleted adult albino rats ... 65
FRENCH, CYRUS E., AND FREDERICK J. STARE. Nutritional surveys in western Holland: Rotterdam 1945 649

GALACTOSE or lactose, excessive quantities of, diets containing, young rats on, nutritional failure, biochemical defect underlying ... 221
GEYER, BEVERLY R. See Geyer, Geyer, Derse, Zinkin, Elvehjem and Hart 129
—— ROBERT P., BEVERLY R. GEYER, PHILIP H. DERSE, TAYA E. ZINKIN, CONRAD A. ELVEHJEM AND EDWIN B. HART. Growth studies with rats kept under conditions which prevent coprophagy ... 129
GOYCO, JOSÉ A., AND CONRADO F. ASENJO. The net protein value of food yeast ... 593
Grinding, source, particle size, and method of, effect of. Nutritive value of keratin. III. ... 673
GROSCHKE, ALBERT C. See Bird, Rubin and Groschke ... 319
Growing guinea pigs, surface area and metabolism of ... 331
Growth, chick, linseed protein for, amino acids required to supplement ... 313
—— mortality and recovery in weanling rats maintained on restricted calories. Effect of fat level of the diet on general nutrition. II. ... 583
—— of the odontoblasts of the incisor tooth as a criterion of the vitamin C intake of the guinea pig ... 491
—— promoting action of vaccenic acid 359
—— —— factor for rats present in summer butter ... 339
—— reproduction and physical capacity of rats receiving diets containing various levels of cottonseed oil or margarine fat ad libitum. Effect of fat level of the diet on general nutrition. I. ... 569
—— value of meat and peas, alone or in combination, as a source of protein for ... 469
GUERRANT, NOLLIE B. See Ellenberger, Guerrant and Fardig ... 39
Guinea pig, rat and rabbit, copper and manganese storage in ... 143
—— —— vitamin C intake of, growth of odontoblasts of incisor tooth as a criterion of ... 491
—— pigs, growing, surface area and metabolism of ... 331

HAHN, PAUL F. See Darby, Hahn, Kaser, Steinkamp, Densen and Cook ... 107
HAINES, J. ESTELLE, ANN M. KLOSTERMAN, HAZEL M. HAUCK, MARGARET A. DELANEY AND ALICE B. KLINE. Tissue reserves of ascorbic acid in normal adults on three levels of intake ... 479
—— —— —— See Klosterman, Haines, Hauck and Kline ... 505
HALDI, JOHN, AND WINFREY WYNN. Work performance respiratory exchange, and certain blood constituents after isocaloric meals of low and high carbohydrate content 287
HALL, GERTRUDE. See Westerman and Hall ... 301
—— WALTER K. See Berg, Pund, Sydenstricker, Hall, Bowles and Hock ... 271
HANDLER, PHILIP. The biochemical defect underlying the nutritional failure of young rats on diets containing excessive quantities of lactose or galactose ... 221
HARRIS, MARY E. See Munks, Kaucher, Moyer, Harris and Macy ... 601
—— PHILIP L. See Hove and Harris 95
—— —— —— WILLIAM J. SWANSON AND KENNETH C. D. HICKMAN. Covitamin studies. VI. Effect of tocopherol supplementation on the output of vitamin A, carotene, and fat by dairy cows ... 411
HART, EDWIN B. See Geyer, Geyer, Derse, Zinkin, Elvehjem and Hart 129
HARVEY, ELLERY H. See Daniel and Harvey ... 429
HAUCK, HAZEL M. Plasma levels and urinary excretion of ascorbic acid in women during the menstrual cycle ... 511
—— —— —— See Haines, Klosterman, Hauck, Delaney and Kline 479
—— —— —— —— Klosterman, Haines, Hauck and Kline ... 505
HEGSTED, D. M., AND JANE WORCESTER. A study of the relation between protein efficiency and gain in weight on diets of constant protein content ... 685
HENDRICK, CORNELIA. See Deuel, Meserve, Straub, Hendrick and Scheer ... 569
HICKMAN, KENNETH C. D. See Harris, Swanson and Hickman ... 411
HOCK, CHARLES W. See Berg, Pund, Sydenstricker, Hall, Bowles and Hock ... 271

Holland, western, nutritional surveys in: Rotterdam 1945 649
HOLT, LUTHER E., JR. See Cox, Mueller, Elman, Albanese, Kemmerer, Barton and Holt 437
HOVE, EDWIN L., AND PHILLIP L. HARRIS. Relative activity of the tocopherols in curing muscular dystrophy in rabbits 95
HUFF, J. W. See Rosen, Huff and Periaweig 561
Human subjects fed eggs or soy beans, amino acids in urine of 209
HUMPHREYS, ELEANOR M. See Benditt, Humphreys, Straube, Wissler and Steffee 85
Hydrolysate, enzymic casein, effect of adding methionine to; nitrogen retention studies on rats, dogs and man 437
Hypoproteinemic rat, plasma protein and erythrocytes in, essential amino acids as a source of. Studies in amino acid utilization. II. 85

IMPROVING the nutritive value of flour. I. The effect of supplementing enriched flour with other B-complex vitamins 301
Inborn characteristics determining the response of chickens to a diet free of animal protein 319
Incisor tooth, odontoblasts of, growth of, as a criterion of vitamin C intake of guinea pig 491
Influence of autoclaving soybean oil meal on the digestibility of the proteins 661
—— —— pteroylglutamic acid (a member of the vitamin M group) on the absorption of vitamin A and carotene by patients with sprue 243
Inositol, pteroylglutamic acid, riboflavin and niacin. Deficiencies of certain vitamins as studied with turkey poults on a purified diet. I. 1
Intake(s), protein, normal and low levels of, effect of methionine upon urinary nitrogen in men at 371
—— three levels of, tissue reserves of ascorbic acid in normal adults on 479
—— varied, of thiamine and vitamin A, conjoint effects of, on rats 459
—— vitamin C, of guinea pig, growth of odontoblasts of incisor tooth as a criterion of 491
Iron, radioactive, absorption of, by children 7–10 years of age 107
Isocaloric meals of low and high carbohydrate content, work performance, respiratory exchange, and certain blood constituents after 287
Isoleucine, leucine, valine and phenylalanine. Amino acid content of feeds. I. 553

JANSEN, B. C. P. See Boer, Jansen and Kentie 339
—— —— —— —— —— Kentie and Knol 359
JOHNSON, BRUCE C. See Wiese, Johnson, Mitchell and Nevens 263
—— RICHARD M., HAREY J. DEUEL, JR., MARGARET G. MOREHOUSE AND JOHN W. MEHL. The effect of methionine upon the urinary nitrogen in men at normal and low levels of protein intake 371
JONES, EDGAR. See Darby, Kaser and Jones 243
JUKES, THOMAS H., EVAN L. R. STOKSTAD AND MARGARET BELT. Deficiencies of certain vitamins as studied with turkey poults on a purified diet. I. Pteroylglutamic acid, riboflavin, niacin and inositol 1

KAPLAN, HATTIE. See Krause and Pierce 633
KASER, MARGARET M. See Darby, Hahn, Kaser, Steinkamp, Densen and Cook 107
—— —— —— —— —— Kaser and Jones 243
KAUCHER, MILDRED. See Munks, Kaucher, Moyer, Harris and Macy 601
KELLY, EUNICE. See Dye, Porter and Kelly 459
KEMMERER, KENNETH S. See Cox, Mueller, Elman, Albanese, Kemmerer, Barton and Holt 437
KENTIE, A. See Boer, Jansen and Kentie 339
—— —— —— —— —— Kentie and Knol 359
Keratin, nutritive value of. III. Effect of source, particle size, and method of grinding 673
Keratosis follicularis, children with, blood levels and absorption of vitamin A in 633
KIBLER, HUDSON H., SAMUEL BRODY AND DOROTHY WORSTELL. Surface area and metabolism of growing guinea pigs 331
KLINE, ALICE B. See Haines, Klosterman, Hauck, Delaney and Kline 479
—— —— —— —— —— Klosterman, Haines, Hauck and Kline 505
KLOSTERMAN, ANN M., J. ESTELLE HAINES, HAZEL M. HAUCK AND ALICE B. KLINE. The renal threshold for ascorbic acid. A modified method for its estimation with results for twelve adult subjects 505
—— —— —— See Haines, Klosterman, Hauck, Delaney and Kline 479
KNOL, H. W. See Boer, Jansen, Kentie and Knol 359
KRATZER, FRANK H., DELBERT WILLIAMS AND ELIZABETH F. BAKER. Amino acids required to supplement linseed protein for chick growth 313
KRAUSE, R. F., AND HAROLD B. PIERCE, with the technical assistance of Ruth Law and Hattie Kaplan. Blood levels and absorption of vitamin A in children with keratosis follicularis 633
KRAYBILL, HENRY R. See Wilder and Kraybill 235

LACTATION and reproduction in highly inbred strains of mice on synthetic diets 703
Lactose or galactose, excessive quantities of, nutritional failure of young rats on diets containing, biochemical defect underlying 221
LAW, RUTH. See Krause and Pierce 633
Lecithin, crude, effect of, on coefficient of digestibility and rate of absorption of fat 177
LEHRER, WILLIAM P., JR., ELLA WOODS AND WILLIAM M. BEESON. The value of meat and peas, alone or in combination, as a source of protein for growth 469

LEMLEY, JANET MCRAE, RAYMOND A. BROWN, ORSON D. BIRD AND ARTHUR D. EMMETT. Absorption and storage of vitamin A in the liver of the rat 53
Leucine, valine, isoleucine and phenylalanine. Amino acid content of feeds. I. 553
Linseed protein for chick growth, amino acids required to supplement 313
Liver of rat, absorption and storage of vitamin A in 53
LOEB, HAROLD G., AND GEORGE O. BURR. A study of sex differences in the composition of rats, with emphasis on the lipid component. Sex difference in susceptibility to essential fatty acid deficiency with high and low fat diets 541
LORENZEN, EVELYN J., AND SEDGWICK E. SMITH. Copper and manganese storage in the rat, rabbit and guinea pig 143
LUECKE, RICHARD W., WARREN N. MCMILLEN, FRANK THORP, JR., AND CAROLYN TULL. The relationship of nicotinic acid, tryptophane and protein in the nutrition of the pig 251
Lysine content of pork luncheon meat, cooking and curing of, effect of . 235

MCGINNIS, JAMES. See Evans, McGinnis and St. John 661
MCGREGOR, MARGARET A., HELEN T. PARSONS AND WILLIAM H. PETERSON. Biotin balance in the albino rat 517
MCMILLEN, WARREN N. See Luecke, McMillen, Thorp and Tull 251
MACY, ICIE G. See Munks, Kaucher, Moyer, Harris and Macy 601
—— —— —— —— Pratt, Munks and Macy 621
Man, rats and dogs, nitrogen retention studies on; effect of adding methionine to an enzymic casein hydrolysate 437
Manganese and copper storage in rat, rabbit and guinea pig 143
Margarine fat or cottonseed oil, diets containing various levels of, rats receiving ad libitum, growth, reproduction and physical capacity of. Effect of fat level of the diet on general nutrition. I. 569
MATTERSON, LLOYD D. See Singsen, Matterson and Scott 13
Meal, soybean oil, influence of autoclaving, on digestibility of proteins 661
Meals, isocaloric, of low and high carbohydrate content, work performance, respiratory exchange, and certain blood constituents after .. 287
Meat and peas, value of, alone or in combination, as a source of protein for growth 469
—— pork luncheon, lysine content of, cooking and curing of, effect of . 235
MEHL, JOHN W. See Johnson, Morehouse and Mehl 371
Melting point, coefficient of digestibility and rate of absorption of various natural and artificial fats as influenced by, comparison of . 187
Men, urinary nitrogen in, effect of methionine upon, at normal and low levels of protein intake . 371
Menstrual cycle, ascorbic acid in women during, plasma levels and urinary excretion of 511
MESERVE, EMILY R. See Deuel, Meserve, Straub, Hendrick and Scheer 569
Metabolism and surface area of growing guinea pigs 331
—— ascorbic acid, of adolescent children, study of 529
—— nitrogen, amino acids in, with particular reference to role of methionine 389
—— of women during the reproductive cycle. XII. Effect of multivitamin supplements on the vitamin C utilization of nursing mothers 621
—— —— —— —— —— —— —— XI. Vitamin C in diets, breast milk, blood and urine of nursing mothers 601
Methionine-deficient rat, cornea of, formation of capillaries and other tissue changes in 271
—— effect of adding, to an enzymic casein hydrolysate; nitrogen retention studies on rats, dogs and man 437
—— —— —— upon urinary nitrogen in men at normal and low levels of protein intake 371
—— protein, fat, choline, cystine, nicotinic acid and tryptophane, some physiological relationships of 155
—— role of, amino acid in nitrogen metabolism with particular reference to 389
—— some effects of, on utilization of nitrogen in adult dog 361
Mice, albino, use of, to determine utilization of calcium of dehydrated carrots and cabbage 195
—— highly inbred strains of, reproduction and lactation in, on synthetic diets 703
Milk, breast, diets, blood and urine, vitamin C in, of nursing mothers. Metabolism of women during reproductive cycle. XI. 601
MITCHELL, HAROLD H. See Wiese, Johnson, Mitchell and Nevens 263
MOREHOUSE, MARGARET G. See Johnson, Deuel, Morehouse and Mehl 371
Mortality, growth and recovery in weanling rats maintained on restricted calories. Effect of fat level of the diet on general nutrition. II. 583
—— weight loss and recovery in young adult rats maintained on restricted calories. Effect of fat level of diet on general nutrition. III. 641
Mothers, nursing, diets, breast milk, blood and urine of, vitamin C in. Metabolism of women during reproductive cycle. XI. 601
Mouse, dietary zinc deficiency in, some effects of 27
MOYER, ELSIE Z. See Munks, Kaucher, Moyer, Harris and Macy 601
MUELLER, ARTHUR J. See Cox, Mueller, Elman, Albanese, Kemmerer, Barton and Holt 437
Multi-vitamin supplements, effect of, on vitamin C utilization of nursing mothers. Metabolism of women during reproductive cycle. XII. 621

MUNKS, BERTHA, MILDRED KAUCHER, ELSIE Z. MOYER, MARY E. HARRIS AND ICIE G. MACY. Metabolism of women during the reproductive cycle. XI. Vitamin C in diets, breast milk, blood and urine of nursing mothers 601
—— —— See Pratt, Munks and Macy 621
Muscular dystrophy, relative activity of tocopherols in curing, in rabbits 95

NATURAL and artificial fats, various, coefficient of digestibility and rate of absorption of, comparison of, as influenced by melting point 187
Net protein value of food yeast 593
NEVENS, WILLIAM B. See Wiese, Johnson, Mitchell and Nevens 263
NEWELL, C. W., AND CONRAD A. ELVEHJEM. Nutritive value of keratin. III. Effect of source, particle size, and method of grinding 673
Niacin, pteroylglutamic acid, riboflavin and inositol. Deficiencies of certain vitamins as studied with turkey poults on a purified diet. I. 1
—— tryptophane relationships in rats. B_6-deficiency in, role of 561
Nicotinic acid, dietary, requirement of rat for, relation of corn to 169
—— —— protein, fat, choline, methionine, cystine and tryptophane, some physiological relationships of 155
—— —— tryptophane and protein, relationship of, in nutrition of the pig 251
Nitrogen metabolism, amino acids in, with particular reference to role of methionine 389
—— retention studies on rats, dogs and man; effect of adding methionine to an enzymic casein hydrolysate 437
—— urinary, in men at normal and low levels of protein intake, effect of methionine upon 371
—— utilization of, some effects of methionine on, in adult dog 361
Normal adults on three levels of intake, ascorbic acid in, tissue reserves of 479
Nursing mothers, diets, breast milk, blood and urine of, vitamin C in. Metabolism of women during reproductive cycle. XI. 601
—— —— effect of multi-vitamin supplements on vitamin C utilization of. Metabolism of women during reproductive cycle. XII. 621
Nutrition, general, effect of fat level of diet on. II. Growth, mortality and recovery in weanling rats maintained on restricted calories 583
—— —— —— —— —— —— —— —— —— —— I. Growth, reproduction and physical capacity of rats receiving diets containing various levels of cottonseed oil or margarine fat ad libitum 569
—— —— —— —— —— —— —— —— —— —— III. Weight loss, mortality and recovery in young adult rats maintained on restricted calories 641
—— of the pig, nicotinic acid, tryptophane and protein in, relationship of 251
—— poultry, phosphorus in. III. Relationship between source of vitamin D and utilization of cereal phosphorus by the poult 13
Nutritional failure, biochemical defect underlying, of young rats on diets containing excessive quantities of lactose or galactose 221
—— surveys in western Holland: Rotterdam 1945 649
—— value, some observations on, of dialyzed whey solids 429
Nutritive value of flour, improving. I. Effect of supplementing enriched flour with other B-complex vitamins 301
—— —— —— keratin. III. Effect of source, particle size, and method of grinding 673

OBSERVATIONS, some, on nutritional value of dialyzed whey solids 429
Odontoblasts of incisor tooth, growth of, as criterion of, vitamin C intake of guinea pig 491
Oil, cottonseed, or margarine fat, diets containing various levels of, rats receiving ad libitum, growth reproduction and physical capacity of. Effect of fat level of diet on general nutrition. I. 569
—— meal, soybean, influence of autoclaving, on digestibility of proteins 661
On the growth-promoting factor for rats present in summer butter 339

PARSONS, HELEN T. See McGregor, Parsons and Peterson 517
Particle size, source, and method of grinding, effect of. Nutritive value of keratin. III. 673
Peas and meat, value of, alone or in combination, as a source of protein for growth 469
PERLZWEIG, W. A. See Rosen, Huff and Perlzweig 561
PETERSON, WILLIAM H. See McGregor, Parsons and Peterson 517
Phenylalanine, leucine, valine and isoleucine. Amino acid content of foods. I. 553
Phosphorus in poultry nutrition. III. Relationship between the source of vitamin D and the utilization of cereal phosphorus by the poult 13
Physical capacity, growth and reproduction of rats receiving diets containing various levels of cottonseed oil or margarine fat ad libitum. Effect of fat level of the diet on general nutrition. I. 569
Physiological relationships, some, of protein, fat, choline, methionine, cystine, nicotinic acid and tryptophane 155
PIERCE, HAROLD B. See Krause and Pierce 633
Pig, nutrition of, nicotinic acid, tryptophane and protein, relationship of 251
Plasma levels and urinary excretion of ascorbic acid in women during the menstrual cycle 511
—— protein and erythrocytes, essential amino acids as a source of, in hypoproteinemic rat. Studies in amino acid utilization. II. 85
Pork luncheon meat, lysine content of, cooking and curing of, effect of 235
PORTER, THELMA. See Dye, Porter and Kelly 459
Poultry nutrition, phosphorus in. III. Relationship between source of vitamin D and utilization of cereal phosphorus by the poult 13

Poults, turkey, on a purified diet, deficiencies of certain vitamins as studied with. I. Pteroylglutamic acid, riboflavin, niacin and inositol 1
PRATT, J. P., BERTHA MUNKS AND ICIE G. MACY. Metabolism of women during the reproductive cycle. XII. Effect of multi-vitamin supplements on the vitamin C utilization of nursing mothers 621
Protein, animal, diet free of, response of chickens to, inborn characteristic determining 319
—— content, constant, diets of, relation between protein efficiency and gain in weight on, study of 685
—— depleted adult albino rats, mixtures of purified amino acids in, dietary utilization of. Studies in amino acid utilization. I. 65
—— efficiency and gain in weight, relation between, study of, on diets of constant protein content 685
—— fat, choline, methionine, cystine, nicotinic acid and tryptophane, some physiological relationships of 155
—— intake, normal and low levels of, urinary nitrogen in men at, effect of methionine upon 371
—— linseed, for chick growth, amino acids required to supplement 313
—— nicotinic acid and tryptophane, relationship of, in nutrition of the pig 251
—— plasma, and erythrocytes, essential amino acids as a source of, in hypoproteinemic rat. Studies in amino acid utilization. II. 85
—— value, net, of food yeast 593
—— —— of meat and peas, alone or in combination, as a source of, for growth 469
Proteins, digestibility of, influence of autoclaving soybean oil meal on 661
Pteroylglutamic acid (a member of vitamin M group), influence of, on absorption of vitamin A and carotene by patients with sprue 243
—— —— riboflavin, niacin and inositol. Deficiencies of certain vitamins as studied with turkey poults on a purified diet. I. 1
PUND, EDGAR R. See Berg, Pund, Sydenstricker, Hall, Bowles and Hock 271
Purified amino acids, mixtures of, dietary utilization of, in protein-depleted adult albino rats. Studies in amino acid utilization. I. 65
—— diet, turkey poults on, deficiencies of certain vitamins as studied with. I. Pteroylglutamic acid, riboflavin, niacin and inositol 1

QUINN, JEANNE P. See Storvick, Fincke, Quinn and Davey 529

RABBIT, rat and guinea pig, copper and manganese storage in 143
Rabbits, muscular dystrophy in, relative activity of tocopherols in curing 95
Radioactive iron, absorption of, by children 7–10 years of age 107
Rat(s), albino, biotin balance in 517
—— —— protein-depleted adu't, mixtures of purified amino acids in, dietary utilization of. Studies in amino acid utilization. I. 65
—— composition of, study of sex differences in, with emphasis on lipid component. Sex difference in susceptibility to essential fatty acid deficiency with high and low fat diets 541
—— dogs and man, nitrogen retention studies on; effect of adding methionine to an enzymic casein hydrolysate 437
—— growth, reproduction and physical capacity of, receiving diets containing various levels of cottonseed oil or margarine fat ad libitum. Effect of fat level of diet on general nutrition. I. 569
—— —— studies with, kept under conditions which prevent coprophagy 129
—— hypoproteinemic, plasma protein and erythrocytes in, essential amino acids as a source of. Studies in amino acid utilization. II. 85
—— liver of, absorption and storage of vitamin A in 53
—— methionine-deficient, cornea of, formation of capillaries and other tissue changes in 271
—— present in summer butter, on growth-promoting factor for 339
—— rabbit and guinea pig, copper and manganese storage in 143
—— requirmeent of, relation of corn to, for dietary nicotinic acid 169
—— tryptophane-niacin relationships in, B_6-deficiency in, role of 561
—— varied intakes of thiamine and vitamin A on, conjoint effects of 459
—— weanling, maintained on restricted calories, growth, mortality and recovery in. Effect of fat level of diet on general nutrition. II. 583
—— young adult, maintained on restricted calories, weight loss, mortality and recovery in. Effect of fat level of diet on general nutrition. III. 641
—— —— nutritional failure of, biochemical defect underlying, on diets containing excessive quantities of lactose or galactose 221
Rate of absorption and coefficient of digestibility of fat, crude lecithin on, effect of 177
—— —— various natural and artificial fats, comparison of, an influenced by melting point 187
Relation of corn to the requirement of the rat for dietary nicotinic acid 169
Relationship between the source of vitamin D and the utilization of cereal phosphorus by the poult. Phosphorus in poultry nutrition. III. 13
—— of nicotinic acid, tryptophane and protein in the nutrition of the pig 251
Relationships, some physiological, of protein, fat, choline, methionine, cystine, nicotinic acid and tryptophane 155
—— tryptophane-niacin, in rats, B_6-deficiency in, ro'e of 561
Relative activity of the tocopherols in curing muscular dystrophy in rabbits 95
Renal threshold for ascorbic acid. A modified method for its estimation with results for twelve adult subjects 505

Reproduction and lactation in highly inbred strains of mice on synthetic diets 703
—— growth and physical capacity of rats receiving diets containing various levels of cottonseed oil or margarine fat ad libitum. Effect of fat level of diet on general nutrition. I. 569
Reproductive cycle, metabolism of women during. XII. Effect of multi-vitamin supplements on vitamin C utilization of nursing mothers 621
—— —— —— —— —— —— XI. Vitamin C in diets, breast milk, blood and urine of nursing mothers 601
Requirement of rat, relation of corn to, for dietary nicotinic acid 169
Respiratory exchange, work performance, and certain blood constituents after isocaloric meals of low and high carbohydrate content 287
Retention studies, nitrogen, on rats, dogs and man; effect of adding methionine to an enzymic casein hydrolysate 437
REYNOLDS, MAY S. See Steele, Sauberlich, Reynolds and Baumann 209
Riboflavin deficiency in the dairy calf 263
—— pteroylglutamic acid, niacin and inositol. Deficiencies of certain vitamins as studied with turkey poults on a purified diet. I. 1
Role of B_6-deficiency in the tryptophane-niacin relationships in rats 561
ROLLMAN, HILDA S. See Augur, Rollman and Deuel 177
ROSEN, FRED, JESSE W. HUFF AND WILLIAM A. PERLZWEIG. The role of B_6-deficiency in the tryptophane-niacin relationships in rats 561
Rotterdam 1945. Nutritional surveys in western Holland 649
RUBIN, MAX. See Bird, Rubin and Groschke 319

SALMON, WILLIAM D. Relation of corn to the requirement of the rat for dietary nicotinic acid 169
—— —— —— Some physiological relationships of protein, fat, choline, methionine, cystine, nicotinic acid and tryptophane 155
SARMA, PADUBIDRI S., ESMOND E. SNELL AND CONRAD A. ELVEHJEM. The bioassay of vitamin B_6 in natural materials 121
SAUBERLICH, HOWERDE E. See Steele, Sauberlich, Reynolds and Baumann 209
SCHEER, BRADLEY T., DOROTHY F. SOULE, MAX FIELDS AND HARRY J. DEUEL, JR. The effect of fat level of the diet on general nutrition. II. Growth, mortality and recovery in weanling rats maintained on restricted calories 583
—— —— —— JOHN F. CODIE AND HARRY J. DEUEL, JR. The effect of fat level of the diet on general nutrition. III. Weight loss, mortality and recovery in young adult rats maintained on restricted calories 641
—— —— —— See Deuel, Meserve, Straub, Hendrick and Scheer 569
SCHWEIGERT, BERNARD S. Amino acid content of feeds. I. Leucine, valine, isoleucine and phenylalanine 553
SCOTT, HAROLD M. Singsen, Matterson and Scott 13
Seasonal variations in the vitamin A and the carotene content of retail butters 39
SEELEY, ROBERT D. See Allison, Anderson and Seeley 361
Sex differences, study of, in composition of rats, with emphasis on lipid component. Sex difference in susceptibility to essential fatty acid deficiency with high and low fat diets 541
SINGSEN, EDWIN P., LLOYD D. MATTERSON AND HAROLD M. SCOTT. Phosphorus in poultry nutrition. III. The relationship between the source of vitamin D and the utilization of cereal phosphorus by the poult 13
SKIDMORE, BLANCH E. See Day and Skidmore 27
SMITH, SEDGWICK E. See Lorenzen and Smith 143
SNELL, ESMOND E. See Sarma, Snell and Elvehjem 121
Some effects of dietary zinc deficiency in the mouse 27
—— —— —— methionine on the utilization of nitrogen in the adult dog 361
—— observations on the nutritional value of dialyzed whey solids 429
—— physiological relationships of protein, fat, choline, methionine, cystine, nicotinic acid and tryptophane 155
SOULE, DOROTHY F. See Scheer, Soule, Fields and Deuel 583
Soy beans or eggs, human subjects fed, urine of, amino acids in 209
Soybean oil meal, influence of autoclaving, on digestibility of proteins 661
Sprue, patients with, absorption of vitamin A and carotene by, influence of pteroylglutamic acid (a member of the vitamin M group) on 243
ST. JOHN, J. L. See Evans, McGinnis and St. John 661
STARE, FREDRICK J. See French and Stare 649
STEELE, BETTY F., HOWERDE E. SAUBERLICH, MAY S. REYNOLDS AND CARL A. BAUMANN. Amino acids in the urine of human subjects fed eggs or soy beans 209
STEFFEE, CLYDE H. See Benditt, Humphreys, Straube, Wissler and Steffee 85
—— —— —— Frazier, Wissler, Steffee, Woolridge and Cannon 65
STEINKAMP, RUTH C. See Darby, Hahn, Kaser, Steinkamp, Densen and Cook 107
STOKSTAD, EVAN L. R. See Jukes, Stokstad and Belt 1
STONE, MARIE A. See Fenton and Cowgill 703
Storage and absorption of vitamin A in the liver of the rat 53
—— copper and manganese, in rat, rabbit and guinea pig 143
STORVICK, CLARA A., MARGARET L. FINCKE, JEANNE P. QUINN AND BESSIE L. DAVEY. A study of ascorbic acid metabolism of adolescent children 529
Strains of mice, highly inbred, reproduction and lactation in, on synthetic diets 703

STRAUB, EVELYN. See Deuel, Meserve, Straub, Hendrick and Scheer 569
STRAUBE, ROBERT L. See Benditt, Humphreys, Straube, Wissler and Steffee 85
Studies in amino acid utilization. II. Essential amino acids as a source of plasma protein and erythrocytes in the hypoproteinemic rat 85
—— —— —— —— —— I. The dietary utilization of mixtures of purified amino acids in protein-depleted adult albino rats 65
Study of ascorbic acid metabolism of adolescent children 529
—— —— sex differences in the composition of rats, with emphasis on the lipid component. Sex difference in susceptibility to essential fatty acid deficiency with high and low fat diets 541
—— —— the relation between protein efficiency and gain in weight on diets of constant protein content 685
Subjects, human, fed eggs or soy beans, urine of, amino acids in 209
Summer butter, rats present in, on growth-promoting factor for 339
Surface area and metabolism of growing guinea pigs 331
Susceptibility to essential fatty acid deficiency, sex difference in, with high and low fat diets. Study of sex differences in the composition of rats, with emphasis on the lipid component 541
SWANSON, PEARL P. See Brush, Willman and Swanson 389
—— WILLIAM J. See Harris, Swanson and Hickman 411
SYDENSTRICKER, VIRGIL P. See Berg, Pund, Sydenstricker, Hall, Bowles and Hock 271
Synthetic diets, highly inbred strains of mice on, reproduction and lactation in 703

THIAMINE and vitamin A, varied intakes of, conjoint effects of, on rats 459
THORP, FRANK, JR. See Luecke, McMillen, Thorp and Tull 251
Threshold, renal, for ascorbic acid. A modified method for its estimation with results for twelve adult subjects 505
Tissue changes, other, and capillaries, formation of, in cornea of methionine-deficient rat 271
—— reserves of ascorbic acid in normal adults on three levels of intake 479
Tocopherol supplementation, effect of, on output of vitamin A, carotene, and fat by dairy cows. Covitamin studies. VI. 411
Tocopherols, relative activity of, in curing muscular dystrophy in rabbits 95
Tooth, incisor, odontoblashts of, growth of, as a criterion of vitamin C intake of guinea pig 491
Tryptophane-niacin relationships in rats, B_6-deficiency in, role of 561
—— nicotinic acid and protein, relationship of, in nutrition of the pig 251
—— protein, fat, choline, methionine, cystine and nicotinic acid, some physiological relationships of 155
Turkey poults on a purified diet, deficiencies of certain vitamins as studied with. I. Pteroylglutamic acid, riboflavin, niacin and inositol 1

URINARY excretion and plasma levels of ascorbic acid in women during the menstrual cycle 511
—— nitrogen in men at normal and low levels of protein intake, effect of methionine upon 371
Urine, amino acids in, of human subjects fed eggs or soy beans 209
—— diets, breast milk and blood, vitamin C in, of nursing mothers. Metabolism of women during reproductive cycle. XI. 601
Use of albino mice to determine the utilization of the calcium of dehydrated carrots and cabbage 195
Utilization, amino acid, studies in. I. Dietary utilization of mixtures of purified amino acids in protein-depleted albino rats 65
—— —— —— —— —— II. Essential amino acids as a source of plasma protein and erythrocytes in the hypoproteinemic rat 85
—— dietary, of mixtures of purified amino acids in protein-depleted albino rats. Studies in amino acid utilization. I. 65
—— of calcium, use of albino mice to determine, of dehydrated carrots and cabbage 195
—— —— cereal phosphorus and source of vitamin D, relationship between, by the poult. Phosphorus in poultry nutrition. III. 13
—— —— nitrogen, some effects of methionine on, in adult dog 361

VACCENIC acid, growth-promoting action of 359
Valine, leucine, isoleucine and phenylalanine. Amino acid content of feeds. I. 553
Value of meat and peas, alone or in combination, as a source of protein for growth 469
Vitamin A, absorption and storage of, in liver of rat 53
—— and carotene, absorption of, influence of pteroylglutamic acid (a member of vitamin M group) on, by patients with sprue 243
—— —— —— content, seasonal variations in, of retail butters 39
—— —— —— thiamine, varied intakes of, conjoint effects of, on rats 459
—— —— blood levels and absorption of, in children with keratosis follicularis 633
—— —— carotene, and fat, output of, effect of tocopherol supplementation on, by dair cows. Covitamin studies. VI. 411
—— B_6, bioassay of, in natural materials 121
—— C in diets, breast milk, blood and urine of nursing mothers. Metabolism of women during reproductive cycle. XI. 601
—— —— intake of guinea pig, growth of odontoblasts of incisor tooth as a criterion of 491
—— —— utilization of nursing mothers, effect of multivitamin supplements on. Metabolism of women during reproductive cycle. XII. 621

—— D, source of, and utilization of cereal phosphorus, relationship between, by the poult. Phosphorus in poultry nutrition. III. 13
—— M group (pteroylglutamic acid), influence of, on absorption of vitamin A and carotene by patients with sprue 243
Vitamins, certain, deficiencies of, as studied with turkey poults on a purified diet. I. Pteroylglutamic acid, riboflavin, niacin and inositol 1
—— other B-complex, effect of supplementing enriched flour with, improving the nutritive value of flour. I. 301

WEANLING rats maintained on restricted calories, growth, mortality and recovery in. Effect of fat level of diet on general nutrition. II. 583
Weight, gain in, and protein efficiency, relation between, study of, on diets of constant protein content 685
—— loss, mortality and recovery in young adult rats maintained on restricted calories. Effect of fat level of diet on general nutrition III. 641
WESTERMAN, BEULAH D., AND GERTRUDE HALL. Improving the nutritive value of flour. I. The effect of supplementing enriched flour with other B-complex vitamins 301
Whey solids, dialyzed, nutritional value of, some observations on 429
WIESE, ALVIN C., BRUCE C. JOHNSON, HAROLD H. MITCHELL AND WILLIAM B. NEVENS. Riboflavin deficiency in the dairy calf 263
WILDER, OLIVER H. M., AND HENRY R. KRAYBILL. Effect of cooking and curing of lysine content of pork luncheon meat 235
WILLIAMS, DELBERT. See Kratzer, Williams and Baker 313
WILLMAN, WANDA J. See Brush, Willman and Swanson 389
WISSLER, ROBERT W. See Benditt, Humphreys, Straube, Wissler and Steffee 85
—— —— —— —— Frazier, Wissler, Steffee, Woolridge and Cannon 65
WOODS, ELLA. See Lehrer, Woods and Beeson 469
WOOLRIDGE, ROBERT L. See Frazier, Wissler, Steffee, Woolridge and Cannon 65
Women, ascorbic acid in, plasma levels and urinary excretion of, during menstrual cycle 511
—— metabolism of, during reproductive cycle. XII. Effect of multivitamin supplements on the vitamin C utilization of nursing mothers 621
—— —— —— —— —— —— XI. Vitamin C in diets, breast milk, blood and urine of nursing mothers 601
WORCESTER, JANE. See Hegsted and Worcester 685
Work performance, respiratory exchange, and certain blood constituents after isocaloric meals of low and high carbohydrate content 287
WORSTELL, DOROTHY. See Kibler, Brody and Worstell 331
WYNN, WINFREY. See Haldi and Wynn 287

YEAST, feed, net protein value of 593

ZINC deficiency, dietary, some effects of, in the mouse 27
ZINKIN, TAYA E. See Geyer, Geyer, Derse, Zinkin, Elvehjem and Hart 129

NOTICE TO CONTRIBUTORS

THE JOURNAL OF NUTRITION, a copyrighted periodical, appears monthly for the publication of original research bearing on the subject of nutrition and occasional reviews of the literature dealing with this subject.

THE JOURNAL OF NUTRITION is the official organ of the American Institute of Nutrition organized for the dissemination of scientific knowledge regarding nutrition. The officers of the Institute are: A. H. Smith, President; R. M. Bethke, Vice-President; H. E. Carter, Secretary; E. M. Nelson, Treasurer; C. A. Elvehjem, D. W. Woolley and H. J. Almquist, Councillors.

Preliminary notices will not be accepted. No paper which has already appeared will be accepted nor will simultaneous publication elsewhere be permitted. The number of pages in each issue is limited. Therefore, unusually long papers that would take a disproportionate part of a single issue cannot be accepted.

The paper must be accompanied by an author's abstract not to exceed 225 words in length, which will appear on the advance abstract cards of the Bibliographic Service of The Wistar Institute in advance of complete publication. Nothing can be done with the manuscript until the abstract is received.

Manuscripts and drawings should be sent by express prepaid or by registered mail to the Editor, DR. GEORGE R. COWGILL, Yale University Nutrition Laboratory, 333 Cedar Street, New Haven 11, Conn. If two copies of the paper can be submitted, consideration by the Journal can be expedited.

Manuscripts should be typewritten in double spacing on one side of paper 8½ × 11 inches, and should be packed flat — not rolled or folded. The original, not carbon, copy should be sent. The original drawings, not photographs of drawings, should accompany the manuscript. When photographs are used for halftone reproduction glossy prints should be sent. Authors should indicate on the manuscript the approximate position of text figures. If there are illustrations so large as to require mailing separately, reduced copies of them that can be mailed with the manuscript should be provided in order to expedite consideration of the paper.

Manuscripts and drawings should be submitted in complete and finished form with the author's complete address. All drawings should be marked with the author's name. The Wistar Institute reserves the privilege of returning to the author for revision approved manuscript and illustrations which are not in proper finished form for the printer. When the amount of tabular and illustrative material is judged to be excessive or unusually expensive, authors may be requested to pay the excess cost.

The tables, quotations (extracts of over five lines), and all other subsidiary matter usually set in type smaller than the text, should be typewritten on separate sheets and placed with the text in correct sequence. Footnotes should not be in with the text (reference numbers only), but typewritten continuously on separate sheets, and numbered consecutively. Explanations of figures should be treated in the same manner, and, like footnotes, should be put at the end of the text copy. A condensed title for running page headline, not to exceed thirty-five letters and spaces, should be given. Citations of literature should be made in the text by numerals indicating the year of publication. Authors' names (followed by year, title, etc.) should be arranged alphabetically in a list at the end of the paper only.

Figures should be drawn for reproduction as line or halftone engravings, unless the author is prepared to defray the additional cost of a more expensive form of illustration. In grouping the drawings it should be borne in mind that, after the reduction has been made, text figures are not to exceed the dimensions of the printed matter on the page, 4½ × 6½ inches. Single plates may be 5 × 7½ inches, or less. No double plates will be accepted.

Figures should be numbered from 1 up, beginning with the text figures and continuing through the plates. The reduction desired should be clearly indicated on the margin of the drawing.

All drawings intended for photographic reproduction either as line engravings (black-ink pen lines and dots) or halftone plates (wash and brush work) should be made on white or blue-white paper or bristol board — not on cream-white or yellow-toned. Photographs intended for halftone reproduction should be securely mounted with colorless paste — never with glue, which discolors the photograph.

More explicit directions on the preparation of text and illustrations are available in The Wistar Institute Style Brief (The Wistar Institute Press, 1934).

Galley proofs and engraver's proofs of figures are sent to the author. All corrections should be clearly marked thereon.

The journal furnishes the author fifty reprints, with covers, of the paper gratis. Additional copies may be obtained according to rates which will be sent the author as soon as the manuscript has been examined at The Wistar Institute, after acceptance.

THIS NUMBER COMPLETES VOLUME 33

THE JOURNAL OF NUTRITION

VOL. 33 JUNE 10, 1947 No. 6

CONTENTS

BERTHA MUNKS, MILDRED KAUCHER, ELSIE Z. MOYER, MARY ELLEN HARRIS AND ICIE G. MACY. Metabolism of women during the reproductive cycle. XI. Vitamin C in diets, breast milk, blood and urine of nursing mothers 601

J. P. PRATT, BERTHA MUNKS AND ICIE G. MACY. Metabolism of women during the reproductive cycle. XII. The effect of multi-vitamin supplements on the vitamin C utilization of nursing mothers 621

R. F. KRAUSE AND HAROLD B. PIERCE. With the technical assistance of Ruth Law and Hattie Kaplan. Blood levels and absorption of vitamin A in children with keratosis follicularis. Two figures 633

BRADLEY T. SCHEER, JOHN F. CODIE AND H. J. DEUEL, JR. The effect of fat level of the diet on general nutrition. III. Weight loss, mortality and recovery in young adult rats maintained on restricted calories 641

CYRUS E. FRENCH AND FREDERICK J. STARE. Nutritional surveys in western Holland: Rotterdam, 1945. One figure 649

ROBERT JOHN EVANS, JAMES MCGINNIS AND J. L. ST. JOHN. The influence of autoclaving soybean oil meal on the digestibility of the proteins ... 661

G. W. NEWELL AND C. A. ELVEHJEM. Nutritive value of keratin. III. Effect of source, particle size, and method of grinding 673

D. M. HEGSTED AND JANE WORCESTER. A study of the relation between protein efficiency and gain in weight on diets of constant protein content. Ten figures 685

PAUL F. FENTON AND GEORGE R. COWGILL. With the technical assistance of Marie A. Stone 703

PRESS OF
THE WISTAR INSTITUTE
OF ANATOMY AND BIOLOGY
PHILADELPHIA

Printed in the United States of America